QUARTERLY PROGRESS REPORT

RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES, NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND APPROACHES TO ENHANCE NAVY/MARINE CORPS PERSONNEL COMBAT PERFORMANCE

GEO-CENTERS, INC.

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RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES, NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND APPROACHES TO ENHANCED NAVY/MARINE CORPS PERSONNEL COMBAT PERFORMANCE

I. INTRODUCTION

This report summarizes the results of GEO-CENTERS' technical activities for the first quarter of the contractual base year for the Naval Medical Research Institute (NMRI) under Contract N00014-95-D-0048, Delivery Order #001. This delivery order encompasses a variety of scientific studies that are capable of supporting ongoing and projected programs under the cognizance of NMRI, NMRI TOX/DET-Dayton, OH, NDRI-Great Lakes, IL and the NDRI Detachment-Bethesda, MD.

The format for these periodic technical progress reports consists of four sections: (1) Descriptions of work to be performed, (2) Objectives planned for the current reporting period, (3) Significant results, and (4) Objectives for the next reporting period. Accumulated scientific reports, technical reports and journal articles will be provided as part of the annual technical progress reports. Specifically, the research conducted by GEO-CENTERS during this quarterly reporting period has been focused on the following general scientific programs:

1. Infectious disease threat assessment and enterics programs.

2. Toxicological studies.

3. Immune cell biology, wound repair and artificial blood studies.

4. Biomedical diving programs.

5. Dental related diseases.

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6. Personnel performance enhancement programs, and the

7. Breast Disease Center (requirement established in March 1995 and will be reported in depth during the next quarterly technical progress report for the NMRI contract).

INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

(1) GEO-CENTERS has been tasked to conduct R & D in support of the U.S. Navy Enterics program. We are investigating cytokines which are antigen-nonspecific mediators that play an important role in regulating the immune responses as well as the out come of the disease. Dependent upon the site of production, concentration and concomitant presence of other mediators, a given cytokine may contribute to the pathogenesis of an invasive organism like *Campylobacter jejuni*. In the present study, the role of local cytokines in infection and immunity to *C. jejuni* infection in mice is being evaluated.

(2) In order to know whether animal proteins were expressed in the retrovirally-transformed cerebral microvascular endothelial cells and some cell wall-deficient microorganisms, a Western blot technique was employed in this project. The monoclonal antibody against human serum albumin (HSA) and polyclonal antibody against protein kinase C(PKC) were used as probes.

Anti-HSA antibody is specific for human plasma albumin and recognizes the episode resistant to reduction or denaturation commonly used in immunoblotting techniques or in immunohistochemistry. Cross reactivity is observed with rhesus, baboon and gibbon albumin, but not with marmoset albumin. No cross-reactivities are observed with other animals.

PKC, a family of closely related enzymes, plays a central role in the signal transduction of many short-term responses, such as secretion of neurotransmitter, hormones and enzymes, in muscle contraction and alterations in membrane ionic conductance. In addition, PKC activity has been implicated in the regulation of long-term responses, such as gene expression and cell proliferation. Thus, PKC is a very good representative of important mammalian proteins. Antibody against pan PKC can detect all the subtypes of PKC.

(3) Respiratory Virus outbreaks among Navy and Marine Corps personnel have been prevalent for decades. Population densities and shipboard environment, both in and out of port,

provide excellent conditions for transmission studies. One of the diseases that is being investigated by the GEO-CENTERS' staff is the Peruvian Measles Epidemic that is being seen spreading throughout the world.

(4) GEO-CENTERS has been tasked to support the Navy's Infectious Disease Threat Assessment program. We are developing an experimental model using a mouse strain called Severe Combined Immunodeficiency (SCID) for dengue hemorrhagic fever/Shock syndrome (DSS). The model will be used for testing vaccines and understanding immunology and pathogenesis of these diseases. The primary support of the SCID mice program has been at NMRI however, FY94/95 funding for this program has suffered a significant cut and GEO-CENTERS' investigative team on this program have been transferred to other critical infectious disease programs.

The Septic Shock Research Program is involved in studies into the causes, course, prevention and cures of sepsis and septic shock. Within the SSRP, the new Animal Physiology Branch which GEO-CENTERS is starting up focuses on studying the effects of sepsis on oxygen transport to tissue. Interest in this area stems from the fact that septic patients generally have well oxygenated blood and yet some or all of the tissues appear to become progressively hypoxic. Oxygen transport may be impaired at a number of steps between the lungs and the mitochondria. The first problem to be addressed will be the suspected degradation of the oxygen diffusing capacities of various tissues. Sepsis results in severe tissue edema and this edema may result in significant changes in tissue composition and the pathways followed by oxygen as it moves from the capillaries to the mitochondria. These changes may reduce the oxygen diffusion coefficients of the tissue as well as reducing the solubility of oxygen in the tissue. The consequent reduction in the amount of oxygen which can move through the tissue means that although the blood contains sufficient oxygen it cannot leave the circulatory system quickly enough to maintain mitochondrial supply. Initial studies are being conducted to determine which tissues are being most severely impacted by sepsis-induced edema. Other studies are being established to measure oxygen diffusion coefficients and oxygen solubilities in tissues.

Several additional studies are being established on other aspects of oxygen delivery problems during sepsis. In conjunction with researchers at the University of Pennsylvania's Nuclear Magnetic Resonance Center, we are using NMR to measure the skeletal muscle intracellular oxygen levels by observing the degree of myoglobin deoxygenation. Comparison of normal and septic animals will indicate whether the oxygen problems caused by sepsis are located external to or within the myocytes. Another study will determine if the degree of sepsis can be established by following changes in

respiratory and metabolic parameters that can be measured in the expired air. This offers the advantage of being relatively noninvasive while allowing continuous monitoring of one indicator of patient status. Finally, initial work has begun on determination of the effects of sepsis on the oxygen affinity of hemoglobin.

(5) A portion of the Navy's R&D HIV program involves work in Okinawa and Japan. GEO-CENTERS has been tasked to organize and manage the data pertinent to both the human T-cell leukemia/lymphoma virus type-I (HTLV-I) viral epidemiology project (VEP) based in Okinawa. Additionally, we are to geographically type human immunodeficiency virus isolates in Navy personnel studies, conduct epidemiological analyses of these data.

(6) Additional effort in support of the Rickettsial program has lead GEO-CENTERS to isolate and characterize gamma-interferon inducible genes and their products. This is being done in order to more clearly define their role in the functioning of the immune system.

(7) In addition to the above, the Navy's Infectious Disease Threat Assessment program has tasked GEO-CENTERS to characterize and analyze modified lysine in the surface proteins of *Rickettsia prowazekii* and typhi, as well as to find epitopes on the SPA (surface protein antigen) using pin technology.

TOXICOLOGICAL STUDIES

(1) The specific direction taken by our GEO-CENTERS' investigators at NMRI/TD continues to be focused into two major areas of concern for the U.S. Navy that are related to medical concerns on board military aircraft, surface ships and submarines: (a) methylene chloride metabolism and carbon monoxide and carbon dioxide production in a single rat after nose-only exposure to methylene chloride in a closed circulating chamber and (b) the pulmonary effects of toxic dust and smoke inhalation.

In studying the metabolism of methylene chloride for PB-PK modeling, we have been using a 9.2 liter closed circulating chamber for exposure and measuring the methylene chloride uptake and carbon monoxide production through on line GC. The drawbacks in this system are: large chamber volume, accumulation of expired CO by a single rat takes considerable time to detect concentration and estimation of Carboxyhemoglobin (COHb) can be achieved only at one time point i.e. at the end of the exposure period. To circumvent the above mentioned drawbacks we changed the closed circulating chamber design to nose-only exposure system with 700 ml chamber volume with tail projecting out

of the chamber there by allowing us to withdraw blood from tail vein for COHb and plasma CO_2 estimation at any time point during the six hour exposure.

The pulmonary effects of toxic dust and smoke inhalation research is part of the research program to evaluate the pulmonary toxicity resulting from the inhalation of complex atmospheres that are composed of one or more toxic vapors/gases and which also have high aerosol particle concentration. Recent events have revitalized interest in the comprehensive study of the pulmonary effects of vesicant warfare (CW) agents such as 4-bis (methylchloroethyl) sulfide, more commonly known as mustard gas (HD).

Recent reports (USAMRIID-SP-87-03, and Papirmeister et. al., 1991) show that mortality due to exposure to HD is relatively low (1-2% of those exposed), virtually all HD fatalities can be attributed to pulmonary effects and nearly all those exposed to HD (95%) develop debilitating acute respiratory complications such as acute tracheobronchitis, pneumonia and adult respiratory distress syndrome (ARDS). Characterization of the mechanisms and pathogenesis of HD induced pulmonary injury is complicated by the fact that HD inhalation exposure can be either to HD vapor alone or to the so called "dusty mustard" (dHD) preparation in which HD is delivered to the respiratory tract as a complex of HD vapor and HD adsorbed on respirable aerosol particles.

Studies have shown that pollutant gases or vapors that normally do not reach the distal part of the lung may do so when adsorbed on the surfaces of respirable particles and these complex atmospheres may produce physiological effects not induced by either agent alone (Boren, 1964, and Kilburn and McKenzie, 1978). Therefore dHD compared to HD will have a different distribution, clearance and retention in the lung which could result in a more severe or at least, a time course of the pathogenesis of pulmonary injury. Potentiation of the lethality of inhaled CW agents in rodents delivered in aerosol form has been reported (Wheeler,1946). Enhancement of the dermal toxicity of HD delivered as dHD was also reported in these early investigations.

(2) The GEO-CENTERS' Toxicologists at the NMRI/Toxicology Department have been directed toward the continuation of the design and development of inhalation toxicology exposure facilities and to initiate a series of experiments to investigate the pulmonary toxicity of complex (aerosol and toxic vapor) atmospheres. Before initiation of experiments to fulfill the research objectives of an investigation entitled "The Pulmonary Effects of Toxic Dust and Smoke Inhalation: Mustard Gas and Dusty Mustard Surrogates" several analytical problems required solution. These problems were resolved, an experimental schedule which met the requirements of several investigators was formed and

the first in a series of 6 exposures was successfully completed. Our investigators anticipate completing the full series of studies by the end of the next quarterly reporting period.

(3) One of the many toxicology programs supported by GEO-CENTERS' investigators at the U. S. Air Force Armstrong Laboratory for Toxicology in Dayton, Ohio is "species differentiation in skin penetration". The skin is only a partial barrier, it is of great importance to toxicology as a primary or secondary route of exposure. The structure and composition of each laboratory animal skin varies both from each other and from human skin. Although descriptive studies suggest that species differences in dermal absorption are due to these physical and physiological dissimilarities, there has been no viable attempt to understand the underlying principles which are responsible for the differences. For inhalation of chemicals, physiologically-based pharmacokinetic (PBPK) modeling has proven to be a useful tool to predict blood concentrations and tissue doses in humans based on measurable physiological and biochemical differences. This research project will provide an analogous tool for species extrapolation when the skin is the route of absorption. By careful investigation of the effects of species differences in skin structure on permeability constant, the species extrapolation can be achieved and this is critical for accurate dermal risk assessments for various U.S. Air Force chemicals and will make an important contribution to the field of toxicology in general.

(4) GEO-CENTERS' investigators are continuing to conduct toxicological research on various toxins and their effects on human performance. Specific attention is placed on the environments associated with Navy submarines, aircraft and the contaminants consistent with military operations. Our efforts not only deal with a variety of animal models but are deeply involved in developing Physiologically-based Pharmacokinetic Models so that more realistic simulation of toxic effects can be made. In addition, we have been tasked to study the operant behavior of a variety of animal species during exposure to toxins found in military aircraft, ships, and submarines in order to determine possible performance decrements when humans are exposed to certain toxins.

IMMUNE CELL BIOLOGY, WOUND REPAIR PROGRAM AND ARTIFICIAL BLOOD STUDIES

(1) The Navy's Immune Cell Biology program (ICBP) has continued to task GEO-CENTERS to support the program with expertise in cellular immunology and immunopathology by investigating and developing various forms of immunotherapy for the treatment of infections and the development of hematopoietic reconstitution strategies.

(2) During Operation Desert Storm, the need for rapid detection of biological warfare and infectious agents became apparent. Devices which were commercially produced for the armed services failed to meet the necessary requirements. The greatest problem with the use of these devices was the high rate of false positive results. The mission of our laboratory is the development of rapid detection devices for use by our forces anywhere in the world. The word "rapid" in our sense means results in minutes. Our preliminary results indicate that our present design can produce results as sensitive as current ELISA techniques which can take hours. Our design would also be easy to use in the field. Our devices are based on state of the art immunochemistry reactions using latex beads imbedded onto flow through membranes. The development has to be performed under the FDA's Good Laboratory Practices (GLP's) due the fact that the final product will be considered a diagnostic device.

(3) The U.S. military is most interested in developing a means to better understand and perhaps, accelerate the process and time it takes for a wound to heal. The more complete the process is and the shortest time for the process to reach completion could very well determine the fate of many soldiers and sailors and perhaps the outcome of a battle. GEO-CENTERS' investigators are actively participating in wound healing and repair studies in conjunction with NMRI scientists.

(4) The U.S. Navy R&D program concerning Cytokine Receptors has tasked GEO-CENTERS to study the differential expression of cytokine receptors and/or p30-like genes in cerebral microvascular endothelial cell (PCMV) self-organization system.

DENTAL RELATED DISEASES PROGRAM

(1) GEO-CENTERS has been asked to assist with a major problem in the clinical practice of periodontology. Historically, the greatest oral hygiene problem for military personnel is a variety of periodontal diseases. GEO-CENTERS' primary task is to improve the early detection of the diseases and the differential diagnosis of the individual periodontal diseases by using immunodiagnostics.

Immunodiagnostics require specific antibodies, several of which exist at NDRI. Various polyvalent and monoclonal antibody reagents have been developed for use in immunodiagnostics directed towards *T. denticola sp.* and *P. gingivalis* epitopes, either whole cell antigens or outer surface antigens. In addition to specific antigens, specific proteolytic activities have been reported, the so-called 'trypsin-like 'activity that may be

associated with these pathogens. Nucleic acid based probe diagnostics require the development of specific sequences unique to the desired target. Potential targets may be short sequences derived from these proteolytic genes, from variable regions of the 16S rRNA, or from plasmids isolated from these targeted species.

(2) As part of our continuing R & D support for the Naval Dental School, which is a Detachment of the Naval Dental Research Institute, GEO-CENTERS has been tasked to help establish and maintain a microbiological/immunological research program and to develop cooperative research projects between the NDS and other dental R & D organizations both within and outside the Navy.

BIOMEDICAL DIVING PROGRAMS

(1) GEO-CENTERS has been tasked to support the Navy diving biomedical program by studying the use of hydrogen as a component of breathing gas in deep dives. Specific areas of interest include the development and certification of the new Phase III Hydrogen Training Facility. This will include the manufacture of a sophisticated hydrogen-supported hyperbaric chamber.

(2) In addition to support for the diving program, we are formulating and conducting studies to examine thermal balance in a hyperbaric environment and to define the physiological events that occur during rest as well as exercise in a thermally stressful environment.

(3) GEO-CENTERS is actively studying the effects of the high pressure associated with Navy deep diving operations on the neurological system of the body. Accordingly, our investigators are conducting hyperbaric research testing various diving levels and using mixed gases on various nervous systems of animals prior to the implementation into human research.

(4) GEO-CENTERS has been actively developing diving schedules and recompression schedules via modeling techniques using known algorithms.

PERSONNEL PERFORMANCE ENHANCEMENT PROGRAMS

(1) In support of the Navy/Marine Corps personnel thermal studies, GEO-CENTERS has continued to conduct R & D in thermal science with respect to physiological performance

and operational medicine. These studies are in support of microclimate cooling of shipboard damage control/firefighting personnel.

(2) In our continuing support of the U.S. Navy Submarine Medical Research programs, GEO-CENTERS has been tasked to develop computer programs for the submarine medical vision effort. This includes a variety of computer environments used in reaction time-based psychological experiments, whose goal is to enhance the performance of submarine crew members. In addition, our investigators are tasked to improve the target recognition and identification for submarine sonar crews through improvement of the visual and auditory components of sonar signals and monitors.

(3) The Navy's Special Warfare (SPECWAR) program has tasked GEO-CENTERS to evaluate effectiveness of laboratory and field training program(s) of SPECWAR personnel to recommend future guidelines to optimize performance, minimize injury, and to define and diagnose musculoskeletal injuries during physical conditioning of SPECWAR trainees.

With the increased potential for low-level conflict replacing the threat of large scale warfare, there will be an increasing reliance on special operations forces during military operations. The training of special forces personnel is both expensive and extensive, thus limiting the manpower. These individuals must be capable of performing a wide variety of covert operations in a large number of operational environments. It is therefore critical to minimize the individual performance degradation which may occur during military operations requiring the use of special forces.

BREAST DISEASE CENTER

(1) GEO-CENTERS has been tasked by NMRI to support their newly designated program to enlarge the Navy's understanding of breast diseases, with a specific focus on breast cancer. The specific tasking to GEO-CENTERS is to develop the staffing plan for the R&D specialties needed to identify the programs' research subjects, screen the subjects for related medical history, facilitate the clinical protocol for each subject, collect the data, analyze the data and prepare the findings of the study into publishable documentation.

(2) The milestone schedule for this research effort directs that the Center be initially staffed with radiology technicians and nursing specialists by the middle of April 1995 with the remaining of the 19 staff members to arrive in mid to late summer 1995. The official start date with a full compliment of R&D staff will be approximately 1 September 1995. A complete status report will appear in the second quarterly technical report for the NMRI contract.

II. DESCRIPTION OF WORK TO BE PERFORMED

INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

Pratt, Niu

Campylobacter species are a major cause of diarrheal disease in humans and in particular to military personnel who support the national defense throughout the world. Symptoms of the enteric disease include gastrointestinal pain, fever, and dysentery. The incidence of human infections in travelers and the military has prompted interest in the development of a vaccine against the *Campylobacter* organism. The NMRI Enterics program has taken a molecular approach to the vaccine development by studying the genes involved in post transcriptional modification of *Campylobacter* antigens. We, GEO-CENTERS' scientists, perform molecular biological procedures involved in supporting the goals of the NMRI Enterics department.

Yao

As a Scientist III, Dr. Yao is to perform research projects involved in molecular biology of *Campylobacter spp.* in the Molecular Biology Section of NMRI Enterics Program. Major areas of responsible include:

- Molecular characterization of *cheY* gene of *C. jejuni* 81176
- The effect of iron on the invasiveness of *C. jejuni* 81176
- Molecular characterization of gene encoding iron-binding protein from *C. jejuni* 5013

Jendrek

NMRI, GEO-CENTERS and USAMRIID (Ft. Detrick) work collaboratively to better understand the effects of pathogens of infectious diseases on military personnel. In this capacity Mr. Jendrek conducts fermentations in a BL-3 suite under cGLP regulations and depending upon the organism of the fermentation may also perform some or all of the purification associated with the project. He also has to create all documentation associated with any aspect of his position, including Standard Operating Procedures, Batch Records, and any documentation required for newly installed equipment. Also either installs all new equipment related to his projects or oversees their installation by the technicians sent by the supplier. He must also assist in the Molecular Biology aspects of his position, DNA purification, Plasmid isolation, Electroporation, and other techniques are performed on a regular basis.

Weeks

Also in support of the NMRI and USAMRIID joint programs, Ms. Weeks serves as an associate of the principal investigator for a research program involving pathogenic, molecular, and biochemical analysis of bacteria and their virulence factors. Experimentation requires a knowledge and proficiency of laboratory techniques and procedures for performing biochemical and immunological analyses. Conducts surveys of the scientific literature to develop background data on techniques and formulate approaches for the investigations, develops experimental protocols, defines the objectives and priorities of subsidiary problems, and arranges the details of cooperative investigations with other organizations when necessary. Involves immunological techniques for quantitating microbial antigens and host cellular responses by employing appropriate molecular biochemical and immunochemical techniques to identify and analyze microbial constituents and products associated with host responses to infection. Is responsible for the general administration of the laboratory reagents, solutions, enzymes, and other materials and equipment used in conducting the studies described. Is responsible for the cleanliness and orderliness of working areas, freezers, and refrigerators. Is responsible for the training and orientation of all new laboratory technicians. Organizes and accumulates repositories of bacterial strains, plasmids, enzymes and sera with sufficient documentation of the histories of each. Maintains sufficient stocks of all reagents, supplies, and equipment required for a well organized molecular biology laboratory.

TOXICOLOGICAL STUDIES

Briggs

Dr. Briggs is the Department Manager and Senior Scientist for the GEO-CENTERS research conducted at the Navy Medical Research Institute/Toxicology Detachment. In this capacity he also functions as a toxicologist and the Quality Assurance Officer.

Dr. Briggs assures that the collaborative research resources are available to the NMRI/TD staff to accomplish toxicology research. He assists with the research planning and correlates the GEO-CENTERS resources with the mission of the Toxicology Detachment.

As a toxicologist, he uses his training and experience in conducting and supporting toxicology and risk assessment studies to evaluate the potential of military relevant chemicals to produce human health hazards to the fleet.

He functions as the Quality Assurance Officer (QAO) to help assure the quality and integrity of the data collected and presented by the NMRI/TD research staff.

Bowen, Kimmel, Reboulet

The engineering expertise within the aerosol and inhalation toxicology programs at the NMRI TOX/DET is provided by GEO-CENTERS. There were four major areas of research this reporting period.

- Performance decrement (roto-rod) inhalation exposures.
- Spectrex Fire Extinguishant (SFE) characterization.
- Inhalation exposure chamber analysis.
- Operant/Behavioral inhalation exposures.

Smith

Dr. Smith's duties at NMRI/TD is as a senior scientist in the areas of toxicology, pharmacology, chemistry, biochemistry and anatomy and physiology. He is currently study director for the project investigating the toxic effects of SFE Formulation A and the chemical/physical properties of SFE Formulation A, C and D. As study director for SFE, he is responsible for scheduling and organizing personnel and research. He also takes an active part in the research as scientist/technician. He conducts research in the chemistry and biochemistry via method development of new assays for screening procedure.

Prues

My objective for the past quarter has been to assist in the research efforts ongoing at the NMRI/TD; specifically involving myself with the supportive research efforts of LT James Lindsey, Ph.D. and Eldon Smith Ph.D. Major areas of responsible include:

TMPP Project - The Chemical Trimethylolpropane Phosphate is an organophosphate that is generated from the combustion of phosphate ester-based lubricants. Phosphate ester-based lubricants are common aboard Navy vessels. The probability of exposure to this highly toxic substance as the result of contamination resulting from a shipboard fire is considered likely.

In a study performed by the Neurobehavorial workgroup at this institute TMPP was determined to induce epileptiform seizures. Additional studies seem to suggest that TMPP interacts

antagonistically with the GABA receptor-chloride channel complex. It's exact mechanism of action is however undetermined.

SFE Project - The Navy is currently studying the toxicity of a new fire extinguishing agent to replace the currently used Halon 1301. Halon 1301 is an ozone depleting product used to flood the sealed compartments of ships during fires. Aerosol agents of various formulations of the Spectrex Fire Extinguishant (SFE) must be studied as potentially toxic inhalants and the risk to personnel who might be exposed must be assessed prior to their usage.

Narayanan, T.K., Jung

Primary tasks are to continue research in the areas of: (a) isolating and identifying the metabolites of DBNP and (b) identifying the TMPP receptor in the brain. We have also developed two new fields of research: (1) a cell model project in which to study the toxicological effects of substances on an *in-vitro* system without the use of a whole animal and (2) the detection of the amounts of neurotransmitters found in the brain (this study was done in conjunction with LCDR John Rossi III, Lt. James Lindsey, and Ms. Sue Prues).

Ritchie

Dr. Ritchie's purpose is to assist in all areas of research design, protocol preparation, research supervision, statistical analysis and preparation of scientific papers and abstracts in the area of Neurobehavioral Research at NMRI/TD and the military Tri-Service Toxicology Consortium at WPAFB, OH. During the current quarter, he has continued research in four areas: (a) development and validation of a comprehensive Neurobehavioral Toxicity Assessment Battery (The NMRI/TD NTAB); (b) neurobehavioral effects of exposure to low concentrations of Ozone Depleting Substance replacements of military interest; (c) neurobehavioral effects of exposure to low concentrations of single and mixed combustion gases as might be encountered in military scenarios; and (d) anatomical disposition and effects of trimethylolpropane phosphate (TMPP), a potent neurotoxicant produced through the pyrolysis of synthetic lubricants used in military ships and aircraft.

Martin

Ms. Martin edits all technical reports, manuscripts, proposals, standard operating procedures and other materials for presentation from NMRI/TD's staff (and Tri-Service Toxicology per tasking). She ensures these materials are cleared through the appropriate military channels and also serves as a liaison to procurement personnel in acquiring manuscripts reprints. She ensures these

publications and presentations are documented properly for reference purposes. Ms. Martin maintains and secures NMRI/TD's research files, graphics files and briefing files. She also lends her editorial skills as a representative of NMRI/TD in the Tri-Service Toxicology Program Development Committee. Ms. Martin also ensures that authors are provided with the appropriate guidelines and instructions for formatting their publications. She assists GEO-Center's Senior Scientist in administrative matters per tasking. She is responsible for developing and expanding the technical communication program at NMRI/TD (and Tri-Service Toxicology per tasking).

Ademujohn

Ms. Ademujohn's purpose at NMRI/TD is to provide technical support to various aspects of ongoing on-site projects in neurobehavioral research. During this quarter she has been involved in testing the effects of ODS refrigerants, specifically, Freon-12 (Dichlorodifluoromethane), Halon 1211 and Mixed Gas Exposure Testing (specifically Carbon Monoxide - CO) on animal models via computer-aided qualitative and quantitative methods. She also supervises animal training protocols for the modified Wahmann chamber inhalation studies and roto-wheel studies.

Connolly

Responsible for cataloging print and non-print materials for circulation, establishing circulation policies and practices, ordering and maintaining the serials collection, handling reference questions, providing interlibrary loan assistance, locating needed materials in other local libraries, and responding to requests for reprints.

Walsh

As a Scientist III, she is supporting the GEO-CENTERS' mission in the Armstrong Laboratory as a member of the Hazard Assessment Group. She supports a variety of projects to include combustion, explosive and tools development research.

Dong

As a senior scientist, Dr. Dong investigates the "Species Differences in Skin Penetration" project. She is responsible for the laboratory research to determine the rates of penetration of three model chemicals in three animal species.

Grabau

Dr. Grabau plans and conducts image analysis and neuropathology research on toxicology programs for the Armstrong Laboratory. He is responsible for computer images of skin cross-sections to determine the absorption effects of various toxins on animal tissues.

Geiss

Mr. Geiss has continued the methods and protocols development for the molecular biology laboratory of the Armstrong Toxicology Division. Also, he has conducted research in support of current projects, such as the 60-day trichloroethylene study.

Narayanan, L.

Ms. Narayanan's position, as a scientist at the Armstrong laboratory, is involved in the following research projects. 1) Assay of neurotoxic esterase enzyme activity as a bio-marker for neuropathy in rats exposed to Tri-cresyl phosphate (TCP) and tri-ortho cresyl phosphate (TOCP). 2) Estimation of thyroglobuline (hTg) and reverse triiodothyronine (rT3) in control and trifluroidomethane (CF_3 I) exposed rats using RIA. 3) Measurement of nor-epinephrine; epinephrine; dopamine; 5- hydroxy tryptamine and their major metabolites in different regions of the brain in control and trinitrobenzene (TNB) exposed rats using HPLC. 4) Hepatotoxicity studies of perflurodecanoic acid (peroxisome proliferator) in rats. This research is collaborative in nature with Dr. Nicholas Reo, Department Of Biochemistry and Molecularbiology, Wright State University, Dayton, Ohio.

IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND ARTIFICIAL BLOOD PROGRAM

Artificial Blood Research Group

Mincheff

As a senior scientist, Dr. Mincheff assists in the design and planning of experiments necessary to carry out the objectives of the research task. He independently plans the details of each bench experiment, executes the research with the assistance of technicians, and analyzes the data for presentation and publication as may be appropriate.

Hammett

As a research technician, Mr. Hammett independently carries out or assists in carrying out experiments directed at meeting the research objectives of the task. He analyzes data from each experiment he carries out for discussion with and presentation to principal investigators, maintaining all necessary laboratory records. As needed, he maintains laboratory supplies and equipment for performing the assigned task experiments.

Mesbah-Karimi

As a research technician, Ms. Mesbah-Karimi independently carries out or assists in carrying out experiments designed to meet the research objectives of the task. She analyzes the data from each experiment carried out for discussion with and presentation to principal investigators, maintaining all necessary laboratory records. As needed, she maintains laboratory supplies and equipment for performing the required research tasks.

Trickett

As a research support assistant, Ms. Trickett is responsible for ensuring that the laboratories performing the assigned research tasks have all necessary support, supply, and equipment needs carried out for investigators and technicians performing the assigned research tasks. Any PC software and editorial support needed by the research teams to meet the task objectives are the responsibility of Ms. Trickett as well.

Tsonev

As a senior scientist, Dr. Tsonev, together with other investigators, assists in the design and planning of experiments necessary to carry out the objectives of the research task. He independently plans the details of each bench experiment, executes the research and analyzes the data for presentation and publication as may be appropriate.

Okouchi

As a senior scientist, Dr. Okouchi, together with other investigators, assists in the design and planning of experiments necessary to carry out the objectives of the research task. He independently plans the details of each bench experiment, executes the research and analyzes the data for presentation and publication as may be appropriate.

Li

Dr. Li supports the NMRI enterics program. Following moderate to severe hemorrhagic shock (HS), enteric bacteria may invariably transfer from the bowel to the systemic circulation, producing sepsis and severe complications. While adequate fluid replacement may alleviate the immediately life-threatening symptoms, particularly the loss of enteric barrier integrity may lead to septic complications, multiple organ failure and death. Profound immunosuppression has been seen following HS. We found that oral treatment with supernatant from Con A activated spleen cells containing various crude cytokines markedly reduced the number of enteric bacterial colonies. Both cellular and humoral immune response were elevated in these mice. Our results suggested that cytokines (even crude cytokines) may play significant role in the amelioration of bacterial translocation, either by augmenting the immune responses or by altering the enteric barrier. Using our modified murine HS model, the oral administration of cytokines on the prevention of bacterial translocation from the gut to the systemic circulation has been studied.

Lipopolysaccharide (LPS) from Gram-negative bacteria are considered to be the responsible agents for the induction of endotoxic shock, affecting the liver and intestine as the target organs. The relative cell lines have been selected. We planned to stimulate these cells with various concentrations of LPS and incubated in low oxygen to mimic HS *in vitro*.

Chavez

At the Blood Research Detachment of Walter Reed Army Institute of Research (WRAIR), our mission is to study aspects of blood research. Major foci in this area include basic research on the physical properties of hemoglobin, red cell storage and preservation, blood banking, and multiple levels of clinical trials on hemoglobin-based blood substitutes. My role at WRAIR involves basic research on the properties of hemoglobin and hemoglobin-based blood substitutes and technical assistance for pilot plant operations. Hemoglobin is the protein responsible for oxygen transport. Hemoglobin oxidation, toxicity, and nitric oxide binding are some of the current problems in the field of hemoglobin-based blood substitutes. I have designed several projects in order to more fully understand the mechanisms involved in hemoglobin function. This knowledge will hopefully allow for alleviation or elimination of problems associated with hemoglobin-based blood substitutes and lead to a viable blood substitute product.

DENTAL DISEASES-RELATED RESEARCH

Zablen

Dr. Zablen supports the NDRI peridontal diseases research program. Treatment varies from mechanical removal of plaque and bacteria, or antibiotic dosage, to a combination of mechanical and antibiotic treatment. The importance of accurate identification of the bacterial species is imperative due to differing levels of antibiotic resistance and susceptibility of the known pathogens. Incorrect usage of antibiotics may lead to adventitious overgrowth and infection by resistant pathogens. Here at NDRI efforts are being directed towards developing rapid chair side applications of immunodiagnostics and nucleic acid probe based diagnostics to facilitate the clinician in diagnosis and treatment of periodontitis.

Immunodiagnostics require specific antibodies, several of which exist at NDRI. Various polyvalent and monoclonal antibody reagents have been developed for use in immunodiagnostics directed towards T. denticola sp. and P. gingivalis epitopes, either whole cell antigens or outer surface antigens. In addition to specific antigens, specific proteolytic activities have been reported, the so-called 'trypsin-like' activity that may be associated with these pathogens. Nucleic acid based probe diagnostics require the development of specific sequences unique to the desired target. Potential targets may be short sequences derived from these proteolytic genes, from variable regions of the 16S rRNA, or from plasmids isolated from these targeted species.

The tools of the Molecular Biologist offer a means to facilitate the developments described thus far. The purpose of this study is to provide a variety of rare reagents via recombinant DNA technology to NDRI scientific staff (Microbiology and Immunology).

- Clone and express the outer membrane antigens and other surface antigens of T. denticola sp., T. socranskii sp., and P. gingivalis in E. Coli. Characterize the cloned genes re: sequence, purification of expressed product, antigenicity, and usefulness in immunodiagnostic assays.

- Clone, express and characterize the 'trypsin-like' proteolytic activities of these species. Assess the suitability of these genes as diagnostic markers and as nucleic acid probe targets.

- Screen the known cultivatable oral pathogens at NDRI for plasmid content. Assess how prevalent these plasmids are, in which species do they exist, are they unique, and can they be utilized in a rapid DNA probe assay?

PROS, Pathogen related oral spirochetes, have been identified by Reviere et al as spirochetes associated with ANUG, acute necrotic ulcerative gingivitis. These organisms, uncultivated to date, cross react with specific Mabs directed towards surface antigens of Treponema pallidum, the causative agent of syphilis. Patients with ANUG or PROS elicit an immune response to these antigens, although the patients have no history of syphilis and are seronegative for syphilis. These antigens may serve as a means for an immunodiagnostic test. These two antigens will be cloned to provide recombinant reagents to develop such a test.

Turner

Dr. Turner's research is currently focused on specific host defense factors which may play essential roles in soft tissue disorders of concern to dentistry. Factors synthesized and released by neutrophils into inflamed tissue spaces of special interest and are receiving close attention. These factors, known as lysosomal enzymes, are known to be involved in killing microorganisms, including the organisms believed to be involved in periodontal disorders. We have, and plan to continue to compare lysosomal activity of neutrophils isolated from healthy patients' blood with activities derived from neutrophils isolated from blood of patients suffering from various kinds of periodontal disorders.

Spencer

The preservation of the fighting strength of the Armed Forces is the primary function of health care providers in the military. This function is carried out in two basic ways: the care of the sick & wounded and the prevention of personnel loss because of illness. Due to current scenarios, the need for insuring that every available soldier is present, able, and ready to perform his duty, from a medical standpoint, is more critical than ever before.

Factors which have served to magnify the importance of preventing medically-related lost duty time include the increase intensity of the modern battlefield, the interdependence and sophistication associated with crew-served weapons systems, potential lack or delay of medical evacuation, and the likelihood of limited reinforcements against a numerically superior force. The prevention and treatment of dental disease and emergencies has been, and continues to be, an important factor in maintaining the fighting strength of the military.

The objective of the clinical department is to work on the reduction of dental emergencies, more specifically the "Effects of Gender-Related Factors on the Incidence of Localized Alveolar Osteitis" (AO study).

Lamberts

At the Naval Dental Research Institute, a backlog of potential manuscripts and reports has developed, particularly in the area of biochemistry and immunology. Dr. Lamberts is responsible for providing technical and professional editing for these manuscripts. Although many reports have been given over the past several years at research meetings, such as the International Association for Dental Research (IADR), the abstracts of such meetings are not generally considered part of the open scientific literature. Hence, there has been a need for an editorial consultant to facilitate the preparation and submission of scientific manuscripts for publication.

Ahlf

Ms. Ahlf is a Senior Scientist I/Dental Research Hygienist and as such, performs the following responsibilities:

Acting as liaison and/or clinic monitor for the scientists at the Naval Dental Research Institute and the facilities where research is being conducted. Providing consultation and design techniques for the accomplishment of research with the 60,000 recruits processing through Great Lakes in a year with minimal adversity. Generating research ideas and directions to increase efficiency and to support the mission of combat readiness. Identifying patient population sub sets who will be sufficiently available to complete the study. Designing studies to track subjects at subsequent duty stations. Coordinating the collection of plaque, saliva, crevicular fluid, and blood samples. Evaluating subjects dental health with plaque and gingival indices and chart dental information, utilizing specialized equipment to determine pocket depths, attachment loss and mobility as per research protocol, and in some cases interpret the data. Providing non-surgical periodontal treatment as needed for research purposes. Coordinating in-service programs for the contracting hygienists as well as assisting with calibrating personnel on techniques pertinent to NDRI research protocol. Creating and producing instructional materials.

Miller

Dr. Glenn Miller is a Senior Research Scientist and is primarily responsible for all aspects of Immunological and Microbiological activities within the Naval Dental School. This includes the development and supervision of research protocols, dental resident mentoring activities, instruction of courses in dental microbiology and dental immunology, supervision of various GEO-Centers personnel, serving as a link between NIH sponsored research and Naval Dental Research programs, and troubleshooting of research programs, computers, instrumentation and equipment.

Gu

As a GEO-CENTER's Scientist II, Ms. Gu's responsibilities include various research activities related to the oral biology programs at the Naval Dental School. This includes but is not limited to the development of reverse transcriptase polymerase chain reaction (RT-PCR) technology for identification of T-lymphocyte Vb mRNA; the quantitation of T-lymphocyte Vb mRNA level in adult periodontal diseases or in response to superantigen and anaerobic bacteria treatment. In addition, I collaborate with investigators at the National Institutes of Health (Drs Scott Diehl and Joel Schawartz relative to projects being conducted jointly by GEO-Centers, NDRI, and NIH.

BIOMEDICAL DIVING RESEARCH

Shea

Dr. Shea's position as a senior scientist II at NMRI is primarily directed to the analysis of neurotransmitter in the CNS which are recovered by the technique of microdialysis. This method, when used in various animal models of stress, provides the samples in which different transmitters can be monitored in a living animal over time. A variety of different procedures using high performance liquid chromatography (HPLC) are used in the analysis of many transmitters. It is my responsibility that these methods are established and maintained for the above analyses.

Kerr

Mr. Tony Kerr supports the technical areas of two programs within the NMRI diving medicine department. They are:

ALZHEIMER STUDY - He supports a Veterans Affairs grant funding Alzheimer's Disease research investigating neurotransmitter modulation of cortical B-APP expression in a rat model. He designs and implements protocols for the proposed studies.

DIVING PHYSIOLOGY - He supports an oxygen work unit investigating oxygen toxicity and resulting convulsions. He is specifically involved in setting up microdialysis techniques in a hyperbaric chamber under hyperoxia conditions.

Ruby

Mr. Ruby provides gas analysis support for Navy diving studies at NMRI and develops and develops new gas analysis methods in support of Navy Fleet requirements. Specification, procurement and installation of laboratory chemical analytical instrumentation is also accomplished to support NMRI gas analysis capabilities.

PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

Salander

To extend and enhance the NMRI/Thermal Stress memory research project by contributing expertise in specific scientific areas. To also contribute when surgical procedures are needed for a variety of animal models.

Wolf

Mr. Rick Wolf serves as a medical project manager and provides management support to the Combat Casualty Care Program at the Naval Medical Research and Development Command. Duties include reviewing medical research plans and progress reports, recommending laboratory guidance, evaluating research proposals, drafting periodic and ad hoc management reports and developing presentation materials.

III. TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD:

INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

Niu

Mapping of *Camplyobacter* (wild type and inv$^-$ mutants) chromosomal genes:
- Chromosomal DNA isolation and purification.
- Gel electrophoresis.
- Restriction endonucleases digestions.
- Pulse Field Gel Electrophoresis (PFGE).
- Southern blot.
- Probe the PFGE fragments with ECL method labeled gene markers.

Site specific mutagenesis of the PFGE fragments with Km gene:
- Excise PFGE fragments from low melting gel.
- Endonuclease digestion of the PFGE fragments.
- Self ligation of the digested fragments.
- Cut with second endonuclease.
- Ligated to the Km gene.
- Natural transformation of the mutagenized fragment to wild type *Campylobacter* strain, 81176.
- Grow on the Km selected plates.

Extraction and purification of cloned *Campylobacter* genes:
- Plasmid DNA extraction.
- DNA fragment purification through B-agarase digestion.
- DNA fragment labeled with ^{32}P or ECL.

Complementation of mutant stain with wild type strain DNA:
- Electrophoresis of 81176 (wild type Campylobacter strain) DNA through PFGE (low melting gel).
- Excise the DNA fragment which may contain invasion genes out of PFGE.
- Natural transformation of the DNA to the mutant strain.
- Invasion assay to select the competent stains.

Pratt

Ms. Pratt supports the NMRI Annex program for enterics medicine. The primary focus for Ms. Pratt has been on the characterization and sequence analysis of genes involved in post transcriptional modification of *Campylobacter* antigen "ptmc".

Yao

Molecular characterization of cheY gene of *C. jejuni* 81176:
- To clone the *cheY* gene of *C. jejuni* 81176.
- To construct *cheY* mutant.
- To identify the function of *cheY* protein by chemotactic assay and adherence and invasion assays.
- To sequence the *cheY* gene.

The effect of iron on the invasiveness of *C. jejuni* 81176:
- To perform the adherence and invasion assays of *C. jejuni* 81176 when grown with or without presence of iron.
- To analyze the outer membrane proteins.
- To identify the gene encoding the iron-binding protein by Southern blotting.

Molecular characterization of gene encoding iron-binding protein from *C. jejuni* 5013:
- To clone the gene encoding iron-binding protein from *C. jejuni* 5013.
- To sequence this gene.
- To construct site-specific mutant.

Jendrek

Objectives for Mr. Jendrek to support the collaborative effort between NMRI and USAMRIID for this quarter were to create a final draft of the Batch Record for the Production of Protective Antigen and to perform a fermentation of *Bacillus anthracis* following the batch record. He is also to assist in the purification of the PA produced from the fermentation. He is to repeat fermentation of *Pichia pastoris* BOT-B that had questionable success. He will also need to develop a protocol of Natural Competence for *Bacillus subtillus* and the uptake of DNA.

Weeks

Ms. Weeks also supports the NMRI and the USAMRIID effort. Her primary objectives for this quarter is to map the largest plasmid in *Yersinia pestis*, the pFra plasmid, by using mutant stains of that plasmid. This is to be accomplish by making plasmid preparations of the recombinant strain called CO92, Pst(-) and running cesium chloride purification gradients to isolate the plasmid without any chromosomal contamination.

TOXICOLOGICAL STUDIES

Briggs

Dr. Briggs's supports the NMRI Toxicology Detachment in Dayton, OH and his primary objectives of this quarter were as follows:

- Continued to perform toxicology research support services for the Ozone Depleting Substances (ODS) projects.

- Established a reproductive and developmental toxicology capability at NMRI/TD.

- Continued to establish a Quality Management Program (QMP) at NMRI/TD.

- Responded to tasking by the Management staff at NMRI/TD. This included the preparation and submittal of the Annual Animal Care and Use of Laboratory Animals report to Congress, Standard Operating Procedures, operation plans for support services and research projects, and assist the new O.I.C. with his orientation.

Bowen, Kimmel, Reboulet

The major inhalation and aerosol toxicological engineering objectives for this reporting period were:

Completion of the performance decrement inhalation exposures of test animals to the combustion gas carbon monoxide (CO) in the roto-rod assembly whole-body exposure chamber (Wahmann #4).

Off-site characterization of the proposed aerosol fire extinguishant SFE.

Repair and modification of the NMRI/TD 250 Liter whole-body inhalation exposure chambers.

Preparation for the proposed Operant/Behavioral inhalation exposures of test animals to the ozone depleting substance (ODS) replacement 1,1,1,2 tetrafluoroethane (R-134a).

Smith

Dr. Smith provides senior scientific guidance to the NMRI TOX/DET on biochemistry to work on the Halon replacement program. His goals include:

- Receive Approval from three Animal Use Protocol.
- Conduct a field study on SFE Formulation A at the Chesapeake Beach Detachment of the Naval Research Laboratory.
- Developed standard spectrophotometric assays of acid and alkaline phosphotase for the microplate.
- Bring Room 209 up to NMRI/TD laboratory standards.

Prues

The goals for this period include:

TMPP Project:

The objective of this study is to try to characterize the mechanism of action of TMPP by using In-Vivo microdialysis to obtain timed samples of the neurotransmitters found in normal rat brain. Following determination of a normal baseline concentration of neurotransmitters via electrochemical detection, an i.p. injection of a low dose of TMPP is delivered to the rat and changes observed.

SFE Project:

The Navy is considering replacing it's current aerosol fire extinguishing vehicle Halon 1301, since it has been found to be damaging to the ozone layer of the earth's atmosphere.

Continued Research and Experimentation on new formulations of the SFE are being conducted. Current analysis techniques are being modified so as to expedite data analysis.

Narayanan, T.K., Jung

The objectives for this period were to:

- continue characterization of the TMPP receptor in the brain.
- chromatographically separate the metabolites of DBNP that are found in the urine and feces.
- begin culturing liver cells (WB344) to be used for the cell model experiment.
- determine the growth curve of the liver cells.
- preparation of brain samples for neurotransmitter analysis.

Ritchie

Dr. Ritchie provides neurobehavioral toxicology research for the NMRI TOX/DET: His goals for this quarter include:

NTAB Development:

- Complete operant training (spectral and geometric pattern delayed matching to sample operant schedule tests) in pigeons.
- Implement Progressive Ratio Schedule of operant reinforcement.
- Develop Play Behavior Analysis equipment and implement testing in juvenile rats.
- Develop Porsolt Forced Swim Test (FST) in rats.
- Complete NMRI/TD Roto-Wheel testing in rats.

Ozone Depleting Substance Replacements:

In conjunction with GEO-CENTERS employees Larry Bowen and James Reboulet, technical objectives include continuation of testing and evaluation of the relative toxicity of various Ozone-Depleting Substance replacements (R-134a, etc.). Testing is planned to include NMRI/TD Roto-Wheel, operant schedules and play behavior analysis.

Single and Mixed Combustion Gas Atmospheres:

In conjunction with GEO-CENTERS employees Larry Bowen and James Reboulet, technical objectives include NMRI/TD Roto-Wheel and operant testing of the effects of brief exposure to low concentrations of CO and CO2 in rats and pigeons.

TMPP:

The TMPP Work Unit, redirected to Robert Carpenter (PI) and Eldon Smith (Study Director) in early 1994, was returned to John Rossi III (PI) and Glenn Ritchie (Study Director) in late 1994 by NMRDC directive. Technical objectives include:

- Identification of the TMPP effects on CNS neurotransmitter system resulting in behavioral seizures (with Dr. T.K. Narayanan).
- Investigation of the CNS effects of TMPP exposure using the techniques of microdialysis, flow injection analysis and sequential flow injection analysis (with Dr. James Lindsey and CDR Nathan Lacy).
- Coordination with newly hired electrophysiologist Jan Lin, M.D. to establish a working intra- and extra cellular recording laboratory at NMRI/TD by 1 April 1995. Planned projects include investigation of the effects of TMPP on CNS single cell activities.

Desert Warfare Syndrome:

Was selected by COL Dan Caldwell (U.S. Army) to assist in development of animal housing and neurobehavioral toxicity assessment testing for a 1-3 project wherein rats will be housed for 30 days in environments simulating the physical environments encountered by U.S. Persian Gulf War veterans. Objectives for the quarter include:

- Development of appropriate animal model.
- Development of suitable animal housing enclosure for sub-chronic testing in enclosed Wahmann-like chambers.
- Identification, procurement and validation of shock-delivery equipment to simulate environmental stress.
- Identification of appropriate neurobehavioral tests to evaluate toxicity induced by the sub-chromic exposures.

Martin

As a technical editor for the NMRI TOX/DET, Ms. Martin's objectives for this quarter were to continue to perform the duties outlined in the afore-mentioned position description. Other objectives include preparation for the various conferences in which Tri-Service Toxicology is participating (e.g. Society of Toxicology, NEHC, and Tri-Service Toxicology's April Conference). Finally, her primary objective of this quarter has been to edit, reformat, and recreate an informational package geared toward reservists considering NMRI/TD for their active

duty assignment. This package includes information on the history of Tri-Service Toxicology, descriptions of technical areas, and project descriptions. In addition, this package will include many graphics and photos generated, scanned, and imported into the text in-house.

Binole, Rix

Mr. Binole and Mr. Rix are both computer scientists/programmers in support of the NMRI TOX/DET. The objectives for this period were: (1) Expand our internet presence (2) Complete conversion of our main server to NTAS (3) Continue to seek out and automate lab functions where applicable (4) Provide technical support for in-house staff.

Ademujohn

The major neurobehavioral objectives for our technical staff for this quarter are as follows:

- Testing various ODS and Mixed Gas substances on animal models using diminished capacity as the endpoint in Wistar rats.
- Range finding using roto-wheel-trained animals and measuring different stages of diminished capacity.
- To compile catalog and computerize the above mentioned data.
- To train pigeons for problem solving protocols.
- Daily maintenance of pigeon intake and logging performance results.
- To obtain operant testing and training data for animals used in R134-a Ration Straining exposure testing.
- To organize, catalog and generate computer graphics, cumulatively from the above mentioned data.
- To maintain data for future reference in upcoming publications.
- To be responsible for the procurement of all materials used in testing and training protocols.
- Responsible for documenting and maintaining operant weights.
- Responsible for writing standard operating procedures for pigeon training protocols.
- Responsible for making daily accurate and detailed entries and updates of all work unit laboratory books.
- Responsible for compiling information for and conducting weekly meetings with/between work unit P.I.'s and laboratory technicians.

Connolly

Ms. Connolly provides scientific and technical information services for the NMRI TOX/DET. Her goals for this period include:

- Catalog toxicological and pharmacological materials not yet cataloged.
- Maintain library.
- Provide library service to the toxicology community at WPAFB.

Walsh

Ms. Walsh provides scientific support to the Armstrong Toxicology Laboratory in Dayton, OH with an emphasis on inhalation toxicity of tricresyl phosphate vapor phase lubricant. Goals for this period include:

- Work began on the toxicity characterization of four additional vapor phase lubricants. Tricresyl Phosphate is known to have a toxic ortho isomer and may form other toxic phosphate compounds. These new compounds appear to be less toxic since they do not produce the ortho isomer; however, they must be evaluated for potential phosphate compound formation under use conditions to establish safe exposure levels for humans.

Combustion Toxicity of Advanced Composite Materials:

- Work continued, utilizing the UPITT II method and has been completed to determine the toxicity of the combustion products of B2 composite materials during burning and to assess the toxicity of the off gas products released from previously burned B2 composites.

In Vitro-In Vivo Extrapolation:

- Work began on the tools development program to establish reliable techniques for a rapid *in vitro* screen to test for rank-orders of toxicity and the detection of subtle biochemical changes in primary hepatocytes exposed to toxic compounds.

Dong

Dr. Dong conducts surgical procedures and analyzes dermatological toxic substance studies at the Armstrong Toxicology Laboratory. Her goals for this period include:

- To determine metabolic parameters for Perfluorohexyl Iodide in F-344 rats.
- To measure Chloropentafluorobenzene (CPFB) absorption in Hartley guinea pigs which resulted from dermal exposure of CPFB.
- Poster preparation for 1995 Society of Toxicology annual meeting.
- To write a technical report.

Grabau

Dr. Grabau is a Veterinarian and Pathologist supporting the Armstrong Toxicology Laboratory. His current goals include:

Supported Program Title: Species Differences in Skin Penetration
Position Title and Description: Co-investigator - develop and provide image analysis techniques
Objectives Summary:

> The primary objective for this period was to research methods that would allow quantitation of pathophysiological events associated dermal toxicology research. This was to support the current dermal research group effort determining the feasibility of selected dermal research initiatives, which are being evaluated. A second objective was preparation of a poster presentation (as first author) detailing our developed dermal image analysis research methods for the Society of Toxicology Annual Meeting (March 1995).

Supported Program Title: Combustion Toxicity of Advanced Composite Materials (ACM)
Position Title and Description: Co-investigator - develop and provide image analysis techniques
Objectives Summary:

> Objectives for this period include conducting image analysis on samples obtained from combustion of ACM and preparation for poster presentation (as second author) of this research at the Society of Toxicology Annual Meeting (March 1995).

Supported Program Title: Trichloroethylene (TCE) Biologically Based Health Risk Model
Position Title and Description: Co-investigator - develop and provide image analysis techniques
Objectives Summary:

The principle objective for this period was to conduct image analysis of proliferating cell nuclear antigen (PCNA) and P450 isoenzyme markers in hepatic tissue sections.

Supported Program Title: Intra-Laboratory Research (ILR) Grant - Hepatic Apoptosis in Mice and Rats

Position Title and Description: Title is undetermined - develop and provide image analysis techniques

Objectives Summary:

The objective for this period was to define what future image analysis support will be required.

Geiss

Mr. Geiss conducts scientist I level research for the Armstrong Toxicology Laboratory. His goals include:

- Identify needs in molecular biology research support and design a technical approach to fulfill those needs. This includes developing protocols and research methods for the evaluation of biological effects of Air Force-related materials.

- Cooperate in current research relating to the toxicological effects of trichloroethylene (TCE), its metabolites and other compounds.

- Complete initial hybridization analysis of gerbil brain RNA samples from an experiment involving hyperbaric oxygen (HBO) treatment.

- Train other scientists in molecular biology research methods.

Narayanan, L

Ms. Narayanan supports the Armstrong Toxicology Laboratory and during this quarter, she has focused on the following areas of research:

- Measurement of neurotoxic esterase activity in rats exposed to TCP and TOCP.
- Measurement hTg and rT3 level in control and CF_3I exposed rats using RIA.
- Measurement of neurotransmitters and their major metabolites level in nine different brain regions in control and TNB exposed rats using HPLC.
- Measurement of diacylglycerol level and assay of phospholipase-C activity in control and perflurodecanoic acid treated rat livers.

IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND ARTIFICIAL BLOOD PROGRAM

Artificial Blood Group

The goal for this NMRI-based research team is to achieve an understanding of the mechanism by which the American Red Cross (ARC) red cell storage solutions extend the shelf-life of red blood cells stored at 4°C, and thereby suggest ways by which storage shelf-life times can be further increased to improve blood supply logistics and use for military medical field support operations.

Specifically, the objectives of the program will be to: (1) investigate the mechanism by which the depletion of chloride improves the storage characteristics of red blood cells independent of an influence on pH by altering the rates of production and consumption of ATP and 2,3-DPG; (2) study the red cell energy budget by testing the effects of maneuvers that may mimic chloride depletion effect; and (3) determine the effect of pH during storage and its relationship to the chloride depletion effect.

Li

Dr. Li conducts wound repair research in support of the NMRI mission. Her goals include:

- The establishment of a murine model of HS with indication of blood pressure and to study the preventive effects of cytokine on bacterial translocation from gut to various organs.
- Investigate the role of cytokines in immune response after HS.

- The establishment of cell lines model which mimic the HS *in vitro* in order to investigate cytokine secretion and gene expression in those cells under endotoxic and hypoxic conditions.

Fan

Ms. Fan is a scientist I performing molecular biology research in support of NMRI. Her goals for this period include:

- Continue the investigation of gene expression of nitric oxide synthase (NOS), a major mediator of reduction of cardiovascular muscle contractility during sepsis and endotoxemia, using reverse transcriptase-polymerase chain reaction (RT-PCR) and Southern blot techniques.

- Design PCR primers and internal probes for protein kinase C (PKC), a transducer of nitric oxide synthase activity. Select specific primers and probe for each PKC isotype (alpha, beta-I, beta-II, gamma, delta, epsilon, eta, and zeta). Analyze selected oligonucleotides through GCG gene bank network to ensure their specificity.

- Set up a non-radioisotopic DNA sequencing system in order to analyze the PCR products of PKC mRNA.

- Colaborate with Dr. S. Volgo of Department of Microbiology, Uniformed University of Health Services to investigate gene regulations of cytokines of immune system in cecal ligation and puncture (CLP)-treated mice using RT-PCR and Southern blot.

Chavez

Dr. Chavez is the lead GEO-CENTERS' scientist conducting research in the procedures to enhance the longevity of whole blood and blood components. He works in support of the joint blood research program between NMRI and the Walter Reed Army Institute of Research. The major goal for this quarter was to get the pilot plant in full operation. The pilot plant produces high quality hemoglobin solutions for testing and research. Bionetics was awarded the contract to operate the pilot plant in October 1994 and completed implementation of the required personnel on the first week of December 1994. Training of personnel in the areas of analytical methodologies, cGMP practice, and operation of the pilot plant was given the highest priority. In addition, several assay methods needed to be established and verified, including total

hemoglobin concentration and methemoglobin levels (via absorption spectroscopy), SDS-PAGE and isoelectric focusing gels, and HPLC analysis of the % modified protein.

The research project currently undertaken was a heme affinity experiment with hemoglobin. Hemoglobin contains four protein chains, each enclosing a heme active site where the oxygen binds. To preserve functionality and prevent toxic effects, it is imperative that hemoglobin-based blood substitutes maintain the incorporation of heme within the protein. By using rapid scanning spectrophotometry, one can directly measure the hemoglobin's affinity for heme. By comparing several varieties of heme containing globins, the mechanism by which hemoglobin retains the heme can be found. With genetic engineering and/or chemical modification of the hemoglobin, the optimization of heme retention can be understood, monitored, preserved, and potentially enhanced.

Ring

Dr. Ring's biological research, as in other areas, involves computers for many activities: data organization, communication, etc. However, many of the investigators are not trained in these disciplines. GEO-CENTERS' presence within the Tissue Bank gives the biological investigators a source of help in resolving technical problems. The objectives for the quarter are not hard scientific accomplishments, but rather support functions which contribute to the overall operation of the Immune Cell Biology Program.

The first objective is to provide general computer support. Support for stand-alone computers involves assistance with spreadsheets, databases, and operating systems. Support for network connectivity involves hardware installations, software configuration, and personnel training.

The second objective is to maintain the availability of I.C.B.P.'s calcium imaging system. The system is built around a MicroVaxII main processor with an attached Gould image processor. Software from R.Y. Tsien (University of California) was used as the software base. Numerous hardware and software enhancements have been done. The result is a system highly specialized for the measurement of intracellular calcium. Use of the system has fluctuated, with active periods and slack periods. The objective is to manage the system during the active periods and maintain a "corporate memory" during the slack periods.

The general aim of my work has been and continues to be: how can the tools of computing resources be made available to the biological investigator? In Imaging, networks, and programming, the objective is to be responsive to the needs of individual investigators and the department as a whole.

DENTAL DISEASES-RELATED RESEARCH

Zablen

Dr. Zablen is a senior scientist at NDRI whose quarterly goals include:

- Clone and express the outer membrane antigens and other surface antigens of T. denticola sp., T. socranskii sp., and P. gingivalis in E. Coli. Characterize the cloned genes re: sequence, purification of expressed product, antigenicity, and usefulness in immunodiagnostic assays.

- Clone, express and characterize the 'trypsin-like' proteolytic activities of these species. Assess the suitability of these genes as diagnostic markers and as nucleic acid probe targets.

The objectives above can both be serviced by creating genomic libraries of each pathogenic organism in E. coli utilizing classic recombinant technology. A genomic library can be defined simply as a depository of fragments of a specific genome, representing 299.9% of that genome, in a useful, retrievable, stable form. The most elegant of these library techniques for ease of preparation, use and storage is that of E. coli bacteriophage lambda. Basically, genomic DNA from the target organism is isolated in pure form in a largely intact state, partially digested by endorestriction enzyme treatment to yield DNA size fragments of the appropriate range, and inserted into the lambda vector. A large variety of lambda cloning vectors are commercially available. We have chosen LambdaGEM12 Xhol Half-site Arms from Promega to construct our libraries. This vector allows for low background, high efficiency cloning of genomic libraries. Alternative Lambda cloning vectors will also be considered, such as lambda ZapII, depending on the success rate of the project.

Each library will be screened by the technique of plaque-immunolift using rabbit polyclonal and/or mouse monoclonal antibodies to detect the expression of outer surface antigens. Those positive clones will be purified, characterized, and the genes subcloned into E. coli plasmids for sequencing and expression work up. These same libraries will also be screened for the 'trypsin-like' proteolytic activity associated with these pathogens. The lambda library will be plated and challenged with chromogenic substrates for identification of these enzymatic activities. Positive clones will be processed as described for antigen producing clones.

Reports in the literature indicate some Treponema sp. contain cryptic plasmids which were isolated by standard alkaline lysis procedures. At NDRI we have developed the techniques necessary to successfully screen for plasmid content in all T. denticola strains available. Those plasmids discovered will be characterized re: their usefulness as DNA probes, as will all other cloned sequences demonstrated.

Cloning and expression of Treponema pallidum 47Kd and 37Kd PROS crossreacting antigens in E. coli:

Protocol:

To clone the two T. pallidum Pathogen Related Oral Spirochete (PROS) crossreacting antigens, the polymerase chain reaction (PCR) and the published sequences (Isaacs et al, 1989; Hsu et al, 1989); and Weigel et al, 1992) will be used. Using the sequences from the 5' and 3' ends of the coding region for these polypeptides, PCR primers will be derived containing unique restriction sites to facilitate cloning of the amplified products into E. coli plasmids for characterization and expression.

DNA from T. pallidum, Nichols (T. pallidum cells obtained from T. Fitzgerald) will be amplified in a Perkin-Elmer-Cetus Thermocycler using the Perkin-Elmer-Cetus GeneAmp kit and unique primer oligonucleotides designed to specifically amplify the 47Kd or the 37Kd antigen. Whole cell DNA isolated by standard methodologies, or whole cells boiled in reaction tubes will be the target of amplification. PCR reaction cycles will be optimized to yield maximum product and minimum artifact DNA. Expected amplified products are 1.3Kb for the 47Kd gene and 1.0Kb for the 37Kd gene. Amplified products will be screened by agarose gel electrophoresis.

The resulting amplified DNA will be restricted with the appropriate restriction endonucleases, agarose gel purified, and cloned into the BamHI site of E. coli plasmid pGEX-2T for characterization, sequence verification, and expression. pGEX-2T is especially useful in that a fusion protein is made with glutathione-S-transferase, under ptac control with IPTG induction, which can be purified by column chromatography on glutathione sepharose 4B with cleavage of the fusion by thrombin, yielding the purified protein of interest.

Expressed 37Kd and 47Kd antigen will be identified using T. pallidum specific monoclonal antibodies (Mabs H9-1, H9-2, and C2-1; obtained from S. Lukehart,(Lukehart, et al, 1985) by Western Blot analysis. Clones containing the genes to be expressed will be induced, cell lysate prepared, and SDS-Page performed. The resulting PAGE gels will be transblotted/transferred to membranes, and challenged with the appropriate Mab.

Upon successful completion of the cloning of these antigens, sufficient recombinant antigen will be produced and purified for use in prototype diagnostic assays for PROS antibody in human patient sera.

Turner

Dr. Turner, NDRI senior scientist, will develop assays for the qualitative and quantitative measurement of biological activities expressed by neutrophil lysozymes. Specific enzymatic activity, as well as the microbicidal activity, of these organelles against Treponema denticola and Porphyromonas gingivalis will be measured. In the future, we plan to examine the modulating effects of monocyte secretion factors (cytokines) on neutrophil function.

Spencer

Ms. Spencer conducts technician level R&D at NDRI to support the clinic-based research in oral diseases: Her goals include:

- Complete basic statistics review.
- Complete literature review on menstrual cycle and steroidal contraceptives.
- Complete literature review on alveolar osteitis.
- Assist in the finalization of the paperwork used in the AO study.
- Coordinate and manage the AO study.

Lamberts

Dr. Lamberts is a senior scientific technical editor at NDRI. His objectives this quarter include:

- To assist as an editorial consultant in the preparation or review of manuscripts to be submitted for publication.

- To aid in the preparation of research presentations (such as posters) for scientific meetings, in the review of research proposals, research communications (letters, rebuttals), etc.

Ahlf

Ms. Ahlf is a scientist and dental research hygienist conducting clinical studies at NDRI in support of their oral health programs. Her quarterly goals include:

- Meet the NDRI Personnel, Clinic Administrators, and other key clinic contacts.
- Tour all clinical facilities.
- Acclimate myself to the Navy operation procedures.
- Be cognizant of research possibilities.
- Assist scientists with all facets of their research.
- Learn specialized equipment and instruments in order to collect and evaluate samples.
- Become credentialed to practice dental hygiene on base.
- Become acquainted with the contracting hygienists and organize continuing education meetings.
- Assist with advancement training for the enlisted personnel.
- Participate with TQL training.

Miller

Work Unit: 0601152N.MR00001.001-0063. Evaluation of the influence of superantigens and polyclonal B-cell activators in periodontal disease To continue work on this Individual Research Program and bring it to conclusion by September of 1995. A specific and unique objective for this quarter was to develop and utilize a quantitative reverse transcriptase-PCR procedure to quantitate the expression of T-cell receptor Vb mRNA by human lymphocytes cultured with various extracts from bacteria associated with periodontitis. We have also expanded this project to look for superantigens in Treponema denticola organisms and to ascertain differences in the way lymphocytes from diseased individuals differ from those from non-diseased individuals.

Work Unit: 0601152N.MR00001.001-0063. IL-1 production by polymorphonuclear leukocytes resident in periradicular lesions. To finalize manuscripts and continue evaluation of IL-1 production in inflammatory polymorphonuclear leukocytes utilizing in-situ hybridization methodology to identify specific IL-1 mRNA in cells.

Work Unit: 0601152N.MR00001.001-0063. Long term frozen storage of lymphocytes. To complete writing of a final manuscript encompassing the work done in the first part of this program and to expand the program to include evaluation of cytokine production by lymphocytes after long term frozen storage.

Work Unit 63706N.M0095.006-3014. Clinical evaluation of bacterial leakage of endodontic temporary filling materials. To bring this project to a conclusion with the submission of a manuscript for publication in the Journal of Endodontics.

Work Unit 63706N.M0095.006-3014. Influence of growth factors on gingival and periodontal ligament fibroblasts. To bring this project to completion with the submission of a manuscript to the Journal of Periodontal Research.

To write and submit the following new IR proposals for consideration for funding by NRDC.

- Correlation between protein components in oral secretions and the status of oral health; and

- A Reappraisal of The Role of the Neutrophil In Periodontal and Endodontal Inflammation and Its Relevance For New Diagnostic Procedures

To complete teaching of the Dental Immunology course for periodontal and endodontal residents.

Gu

To develop a RT-PCR technology for identification of T-lymphocyte Vb mRNA. The technique, which included five major steps: 1) the isolation of total RNA from T-lymphocyte obtained from peripheral blood; 2) to synthesize cDNA from the mRNA by reverse transcriptase methodology; 3) to amplify cDNA by using PCR technology; 4) to separate the PCR products on denaturing polyacrylamide gel; and, 5) to analysis the results by using a Genotype software. Following these steps, the conditions for each step will be built up and tested.

To determine and quantify Vb mRNA level in T-cells obtained after pre-culture of cells with various immunological stimulants.

To determine DNA expression in particular oral cancer cells.

Solanki

Continue the progress of two projects (Studies of lymphocytes functions alteration after a long period of freezing, and The influence of T denticola on T cell proliferations) in collaborations with Dental School Residents.

Resume the experiments of freezing study on peripheral blood lymphocytes. Recruit new subjects, controls as well as diseased patients, for experiments dealing with "Influence of T.Denticola on cell proliferation".

Extend this study by stimulating lymphocytes of these subjects with various antigens to observe different proliferation patterns.

Initiate a new study "The generation of superantigen stimulated T cells for identification of VB positive cells" using PCR and flow cytometric procedures to measure the activities of message produced by lymphocytes at the molecular level.

To learn and utilize the Econo Pump System for protein separation process. Along with Econo Pump System, start protein separation of bacterial prep using standard columns.

BIOMEDICAL DIVING RESEARCH

Shea

Establish a two probe microdialysis system in the animal model of Alzheimer's disease. In the last working quarter we were inducing beta amyloid precursor protein (B-APP) in the cortex of rats by injections of the drug NMDA into the nucleus basalis Mynert (NBM). We are now using the contralateral side of the cortex as control for drug effect. Extracellular levels of the neurotransmitter acetylcholine (ACh) have previously been reported by us to correlate inversely with the levels of ß-APP when the NBM receives lidocaine, and microdialysis is performed ipsilaterally in the cortex. Both a sham injection will be done on the non lesioned side as well as microdialysis in the contralateral cortex. Using this system we will determine the effects of injected lidocaine, tetrodotoxin, and NMDA for both ACh and ß-amyloid. Verification of both injection and probe placement will be determined by sectioning frozen brain from these animals after a dye has been perfused at the end of each experiment. The time- course for each drug effect will be characterized.

A statistical analysis will be extensively performed on all the data from the cold induced amnesia studies that were reported in previous quarters.

Information will be gathered on the best methods available for measuring nitric oxide *in vivo*. The objective will be to do these measurements in diving animals as reported last quarter.

Kerr

ALZHEIMER STUDY - The objective for the quarter is the continuation and refining of the subcortical lesion experiments started in June 1994. Subcortical lesions with NMDA to the Nucleus Basalis (NBM) initially causes increases in cortical acetylcholine (ACH), with subsequent decreases over time as the lesion forms. I am plotting a post-lesion time-course for ACH levels and relating it to cortical B-APP levels to determine if B-APP induction is under cholinergic control.

DIVING PHYSIOLOGY - The objective for the quarter is to complete modifications to all the microdialysis equipment and the hyperbaric chamber (as described in the annual progress report), and begin designing experimental protocols for proposed studies.

Porter

- To continue with fleet soda lime analysis as samples come in from the manufacture.
- To continue the testing program for the candidate CO2 analyzers.

Ruby

Assist the Naval Submarine Medical Research Lab (NSMRL) in the selection, acquisition and testing of a portable carbon dioxide analyzer for use in a disabled sub scenario.

Assist the Naval Sea Systems Command (NAVSEA) in the development of a portable carbon dioxide analyzer capable of operation in hyperbaric atmospheres for use in Dry Deck Shelters (DDS) and host ships.

Provide technical support to the cleanvan, the MRCC and the gas farm operations.

PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

Salander

The objectives for this period were to continue an on going training program, FI/FR schedule, for new naive rats. The study designed to examine the effects of CRF and CDAP interactive qualities on the behavior of rats trained on the FI/FR schedule ended. The data for this study is now available to be studied.

Wolf

Become fully integrated in the Combat Casualty Care Department. Become invaluable to the transition between the incumbent and the arriving Research Area manager. Keep the alligators from having any of the Department for lunch.

IV. SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD:

INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

Niu

- Complete the chromosomal mapping of the Campylobacter strain, 81176.
- Localized the invasion gene(s) in the certain PFGE fragment of 81176 strain.
- Site mutagenesis of PFGE fragments of Camplyobacter strain 81176.
- Invasion assay to screen for the competent strains.

Pratt

Since joining the Enterics program in late April, much of my time has been spent learning new procedures and observing the management of the laboratory. I have learned many new techniques used in molecular biology research, and can now employ these techniques independently. Using Quiagen, Applied Biosystems, and Birnbom mini-prep protocols, plasmid extraction and purification is achieved regularly. The ABI 392 DNA/RNA synthesizer has been used to make oligonucleotides, and further processing of the oligos is also performed. Much of my time has been spent performing DNA sequencing and analysis using the ABI 370A Sequencer. Steps including plate set-up, gel pouring, sample preparation, and sequence analysis, are performed weekly. In addition, I perform Southern Blots, Western Blots, Northern Blots, and labeled them with radioactive and non-radioactive methods. I have done Pulse Field gel electrophoresis, run protein and RNA gels, done transformation experiments, and other work, as needed.

Yao

Molecular characterization of *cheY* gene of *C. jejuni* 81176:
- The *cheY* gene of 81176 was cloned and sequenced.
- The *cheY* mutant was constructed.
- The *cheY* gene in involved in chemotaxis not in adherence and invasion.

The effect of iron on the invasiveness of *C. jejuni* 81176:
- Iron is required for adherence and invasion of 81176.
- *C. jejuni* 81176 does not carry the same homologous gene as the iron-binding gene of *C. Coli* VC167.

Molecular characterization of gene encoding iron-binding protein from *C. jejuni* 5013:

- The gene encoding iron-binding protein from *C. jejuni* 5013 was cloned.
- DNA sequencing of this gene is underway.
- The construction of site-specific mutant is underway.

Jendrek

Created a final draft version of the Batch Record for the Production of Protective Antigen and performed two fermentations of *B. anthracis* following his protocol. The PA produced will be going into monkeys for a new vaccine trial. He performed a *Pichia pastoris* BOT-B fermentation for Toxinology, he showed definite growth on methanol. He also developed a protocol for the uptake of DNA by *B. subtillus* through Natural Competence, and he updated the plasmid DNA purification using QIAGEN reagents.

Weeks

The mapping of the above mentioned plasmid has been slow to materialize because this plasmid is a very low copy plasmid due to its size (100 kilobases). Because of this, most of the quarter has been spent making large scale plasmid preparations of the CO92, Pst (-) recombinant strain. During the middle of the quarter, work was also started of the construction of a pFra plasmid library from the above mentioned recombinant strain. The last month of this quarter was spent training a new Army technician. Because she had little experience in the laboratory, training had to start from ground zero. She is currently proficient in setting up and running agarose gels, pouring plates, and performing large scale kit plasmid preps.

TOXICOLOGICAL STUDIES

Briggs

The transition of Command was performed smoothly and with no disruption to the research effort. CAPT Macys had many taskings with GEO-Centers, Inc. staff in completing his duties at NMRI/TD. These were coordinated or performed by the SCR and the transition was accomplished. CDR Still, the current O.I.C. has familiarized himself with the GEO-Centers staff, the projects GEO-Centers Inc. staff are involved with, and the functions of the Institute. Dr. Briggs and many of the Contractor staff have been intimately involved with CDR Still's orientation and familiarization process.

Contract #2728 continues GEO-Centers, Inc. collaboration for the toxicology support services at NMRI/TD. This was accomplished at the beginning of this reporting period. GEO-Centers, Inc. staff have responded to new taskings including the documentation of operating procedures and study plans. These were coordinated by the SCR. This adds to the value of the resources at NMRI/TD in accomplishing the needs of our sponsors.

This quarter was a very active one for the Ozone Depleting Substances project. Dr. Briggs assisted with the protocol development and approval for the new fire suppressant under development. He has assured that resources were in place to conduct the necessary field trials. He also assisted the Study Directors in planning the project activities including assuring the resources are in place for the continuation of the toxicology research.

The project team has held frequent telephone progress sessions and held one meeting in Washington, D.C. for the ODS refrigerant replacement product to be used by the Navy. The purpose of the meeting was to plan for additional toxicology studies during this fiscal year. Dr. Briggs will manage these studies that will be performed within the Tri-Service Toxicology Consortium during the next quarter.

Dr. Briggs went to California to help justify funding for FY95 toxicology studies for the ODS replacement refrigerant. We were successful in securing $3 million dollars to continue the toxicology testing. A portion of this funding will come through NMRI/TD, and Dr. Briggs is in the early stages of developing new models for reproduction and cardiac sensitization.

He monitored toxicology studies twice at Haskell Labs (DuPont), including study and data reviews and a quality assurance audit. He reviewed the reports and/or study data from the acute inhalation studies, acute developmental studies, genetic toxicology studies, the developmental toxicology studies, the 90 day inhalation toxicology studies and the cardiac sensitization study. These studies are essential to achieve EPA approval, which is expected to occur during the next quarter. One candidate will be selected for purchase exclusively by the Navy because it satisfies both the "user friendly " requirements and also satisfies the engineering requirements that are unique to Navy chillers.

Dr. Briggs has performed the necessary arrangements to help develop and validate a quantitative risk assessment model in laboratory animals. Three chemicals that have demonstrated male reproductive system toxicity are to be evaluated to validate this method. Dr. Briggs has communicated with IH staff at NEHC and is an active member of the Navy Reproductive Board, that is establishing a list of chemicals that may cause reproductive system damage to military members. He also attended the EHAC Workshop to assist in establishing a list of potentially

hazardous chemicals in the military environment. This project is funded by SERDP funds, and toxicology studies may be performed at the Tri-Service Toxicology Consortium once the list is approved and the methods for risk assessment are developed.

Dr. Briggs completed and submitted the Annual Report To Congress For Animal Care and Use at NMRI/TD. This Report was a major task as the requirements are becoming more complex and detailed. This information was submitted to NMRI and went to a consultant firm that compiled data from all military institutions that use laboratory animals for research. These data will be reviewed by Congress this Fall.

A Quality Management Program was completed and submitted to the O.I.C. This is the initial step towards compliance with standards used throughout the toxicology community. It is a key essential in our growth and establishment as a Center for Excellence. Dr. Briggs also conducted a data audit on a research program in progress at NMRI/TD, and reported the findings to the Management Council at the Tox Detachment. He also prepared and/or assisted with the preparation of over 40 Standard Operating Procedures during this quarter.

Bowen, Kimmel, Reboulet

The roto-rod apparatus whole body exposure chamber and instruments necessary to analyze exposure atmospheres of CO were prepared. CO calibration curves for a Miran 1ACVF 30-meter cell infra-red spectrometer were obtained by standard gas reference bag preparation. Personal work space industrial hygiene was monitored using calibrated CO detectors (Envirotrack IV^{u}, Quest Technologies, Oconomowoc, WI). A special, in-house generation container was designed, developed and utilized to deliver metered quantities of test material in a safe, consistent manner.

Groups of five test animals were exposed, one at a time, to CO atmospheres ranging from 500 ppm to 4000 ppm. Testing consisted of visual determination of a) loss of equilibrium b) hind limb dysfunction d) fore limb dysfunction and e) loss of righting (incapacitation). At the point of incapacitation, the exposure chamber was rapidly exhausted until baseline conditions were obtained. During this time, recovery was monitored.

Specialized sampling and monitoring apparati were constructed and tested in-house in preparation for field tests to characterize the combustion aerosol formed during pyrolysis of SFE. Characterization took place at the Naval Research Laboratory (NRL) fire testing facility in Bethesda, MD. Cascade impactor samples (In-Tox Products, Albuquerque, NM) were collected to determine mass median aerodynamic diameter (MMAD) and distribution (sg). Filter samples (Gelman 47 mm spectroglass A/E 61631) were collected to determine mass concentration (g/m^3).

Aerosol particle sizer (APS, TSI, Inc., St.Paul, MN) samples were taken to determine MMAD, sg and count median diameter (CMD). A cyclone sampling instrument (In-Tox Products, Albuquerque, NM) was used to gather bulk quantities of test material for specific surface area, density and morphological analyses. Electrostatic precipitator (ESP, In-Tox Products, Albuquerque, NM) samples were collected to determine particle morphology. Gas samples were collected (Enviromax 3000, Liston Scientific) to analyze CO, CO_2 and O_2 levels after pyrolysis. The data that was collected during the field testing is in the process of being analyzed.

An extensive amount of effort by the inhalation team to locate and repair leaks in the NMRI/TD modified Hinner's-type whole body inhalation exposure chambers accounted for much of our time this quarter. Chamber design and construction anomalies were responsible for unacceptable leak rates in all four of the chambers. In their existing state, chamber leak rates ranged from one L/min. (1000 cc/min.) to four L/min. (4000 cc/min.). In an effort to improve both chamber integrity and chamber dynamics (flow distribution), higher quality gaskets (closed cell foam) were implemented; and, inlet and exhaust support lines were secured. Leak checking using dilute chamber concentrations of CFC-12 (dichlorodifluormethane) and a halogen detector (Yokogawa Corp., Newnan, GA) revealed the door to be the major source of leakage. As a result, an interim door constructed of Plexiglas" fitted with lift and turn pawl latches (Southco, Inc., Concordville, PA) was applied. Studies have indicated this design to be acceptable. These repairs and modifications have resulted in an average chamber leak rate of less than 100cc/min (Wahmann #2).

The Operant/Behavioral whole-body inhalation exposure chamber (Wahmann #2) and instruments necessary to analyze atmospheres of R-134a were prepared. It has been proposed that test animals be exposed to atmospheres containing 4% (40,000 ppm) R-134a to study ratio straining. Pending results of data from exposures to this concentration, the next level would be 8% (80,000 ppm). A calibration curve for a Miran 1ACVF 10 cm cell infra-red spectrometer over a range of 3% (30,000 ppm) to 9% (90,000 ppm) was obtained by standard gas reference bag preparation. Personal work space industrial hygiene levels will be monitored using a Miran 1ACVF 30 meter cell infra-red spectrometer point calibrated at 1000 ppm R-134a.

Smith

- Organized and scheduled a field study at the Chesapeake Beach Detachment of the Naval Research Laboratory.
- Started validation of acid phosphatase, alkaline phosphotase and protein microassays.
- Started discussion of developing a new screening procedure for cardiotoxins.

Prues

TMPP Project:

In Vivo on line microdialysis studies of the effects of TMPP on rat brain neurotransmitters involved the following methods:

- Set-up and modification of current instrumentation to accommodate the study.
- Surgical implantation of microdialysis probe guides into the target region of the brain of Sprague Dawley rats.
- Standardization of microdialysis probes.
- Preparation of mobile phase and standard solutions of neurotransmitters for determination of standard curves.
- Data analysis.

SFE Project:

Toxic effects of inhalation exposure to the fire extinguishing agent SFE. SFE is currently of interest to the Navy as it searches for replacements for fire extinguishing agents which have recently been found to be "unfriendly" to the ozone layer. Study methods include:

- Scaling down of commercially available assay methods for use with a microplate reader. The procedures include assays for: ACP, Alp, β-glucuronidase, and Hydroxyproline.
- Data analysis.
- Ordering and organizing laboratory equipment and supplies.

Narayanan, T.K., Jung

TMPP:

The binding of TMPP to receptors in the brain was studied using a competitive binding assay. The $GABA_A$ receptor antagonist ^{3}H-Bicuculline and the agonist GABA were used to determine if TMPP has a strong affinity for the $GABA_A$ receptor. Increasing concentrations of GABA were added to an assay solution that had 0.2 mg/ml of protein and 10^{-9}M of ^{3}H-Bicuculline. This was compared to solutions that contained increasing levels of TMPP.

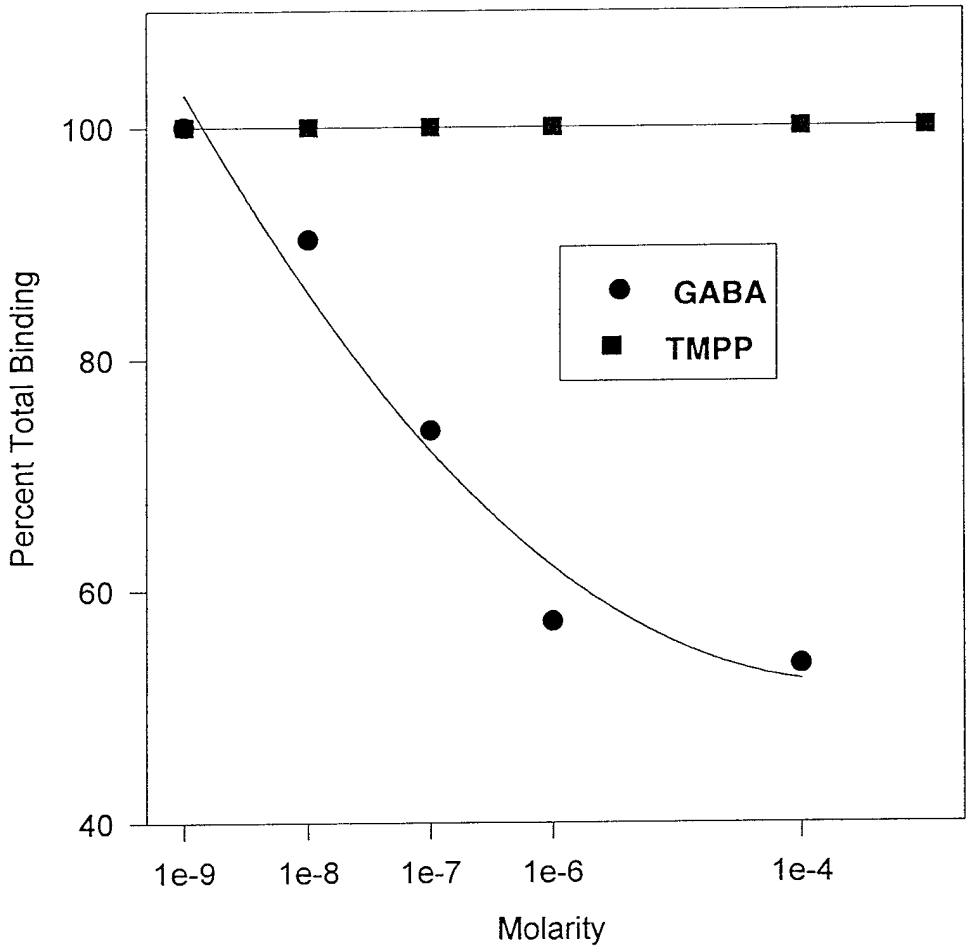

Figure 1. Percent Total Binding to the $GABA_A$ Receptor

The competitive binding studies were performed in a 2 ml system. The antagonist was ^{3}H-Bicuculline (10^{-9}M) with increasing concentrations of agonist GABA (10^{-8}M to 10^{-2}M) and TMPP with approximately 0.20 mg/ml protein in a buffer system consisting of 50 mM Tris and 50 mM NaSCN, pH 7.4. Each binding experiment was done in quadruplicate and the results are plotted as percent binding versus concentration.

The affinity of TMPP for the $GABA_B$ receptor is also being analyzed. ^3H-baclofen is an agonist that was used. Cold baclofen was used to determine the amount of non-specific binding present. A competition assay was run with GABA and TMPP. This showed that TMPP has little affinity for the $GABA_B$ receptor.

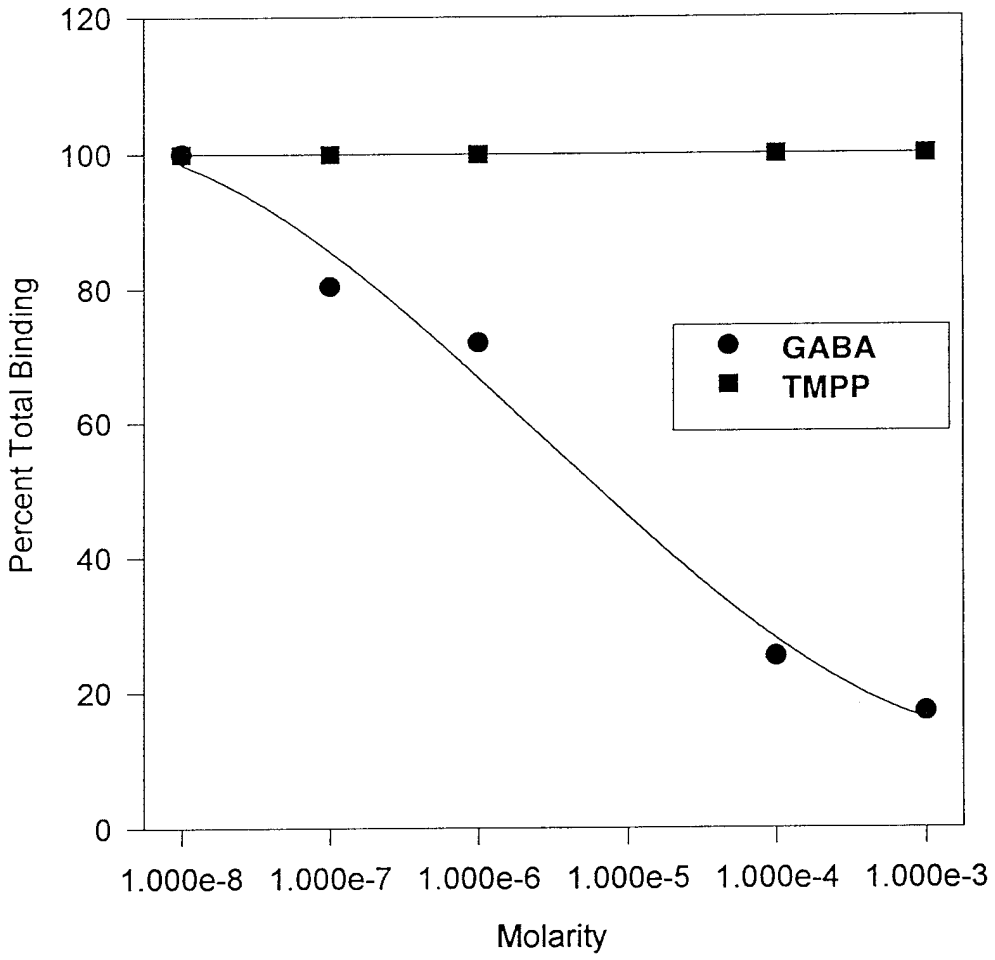

Figure 2. Percent Total Binding to the $GABA_B$ Receptor

The competitive binding studies were performed in a 2 ml system. The agonist used was ^3H-Baclofen (10^{-9}M) with increasing concentrations agonist GABA (10^{-2}M to 10^{-8}M) and TMPP with approximately 0.20mg/ml protein in a buffer system consisting of 286 mM NaCI, 11.8 mM KCI, 5 mM $CaCI_2$, 2.4 mM $MgSO_4$, and 100 mM Tris, pH 7.4. Each binding experiment was done in quadruplicate and results are plotted as percent binding versus concentration.

The clearance rate of TMPP from the blood was measured. A rat was injected with 2 μCi of TMPP i.p. and blood was collected at selected time intervals. The urine was also collected at one hour time points and analyzed for the counts contained therein.

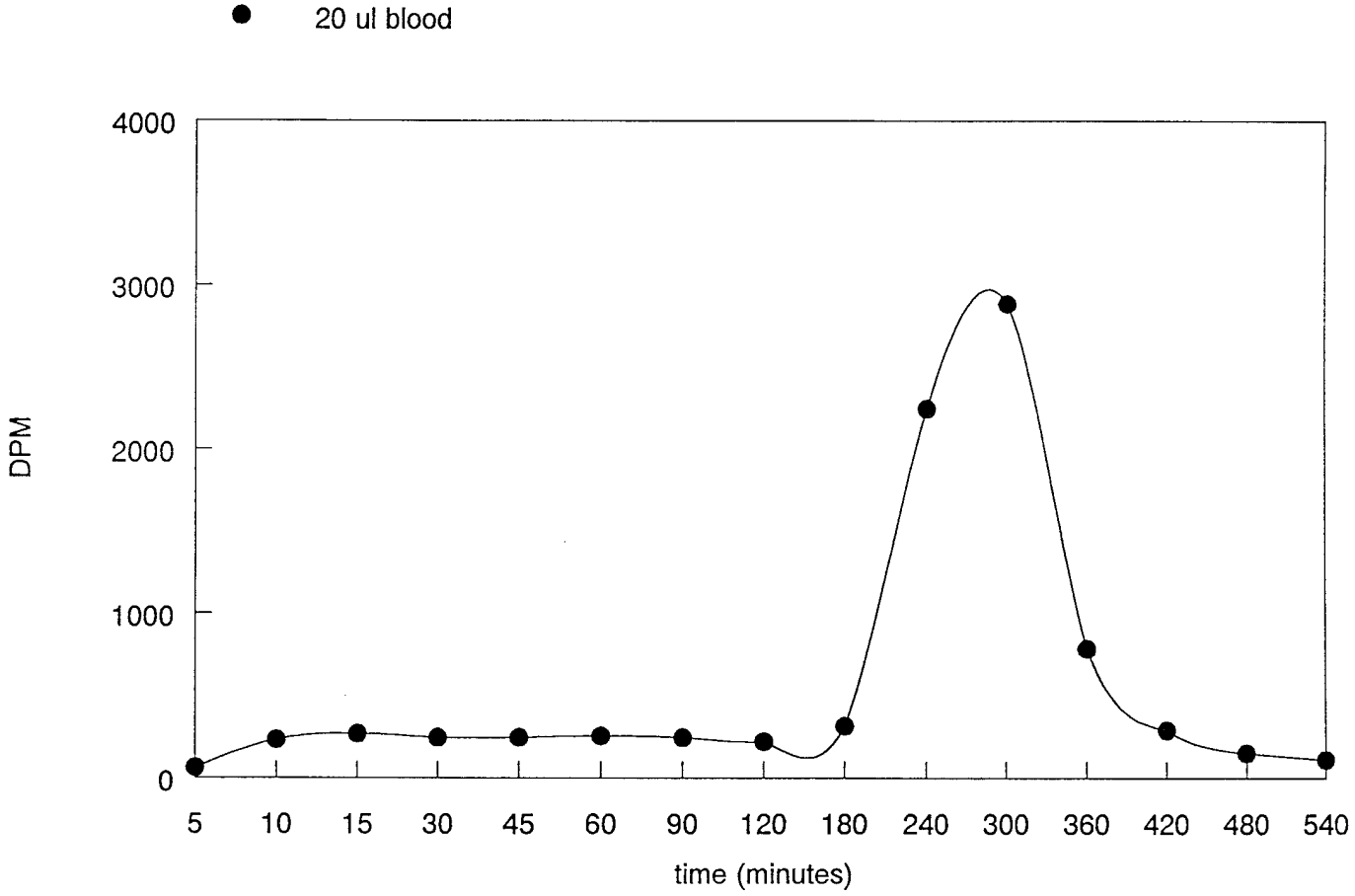

Figure 3. Clearance Rate of TMPP via the Bloodstream

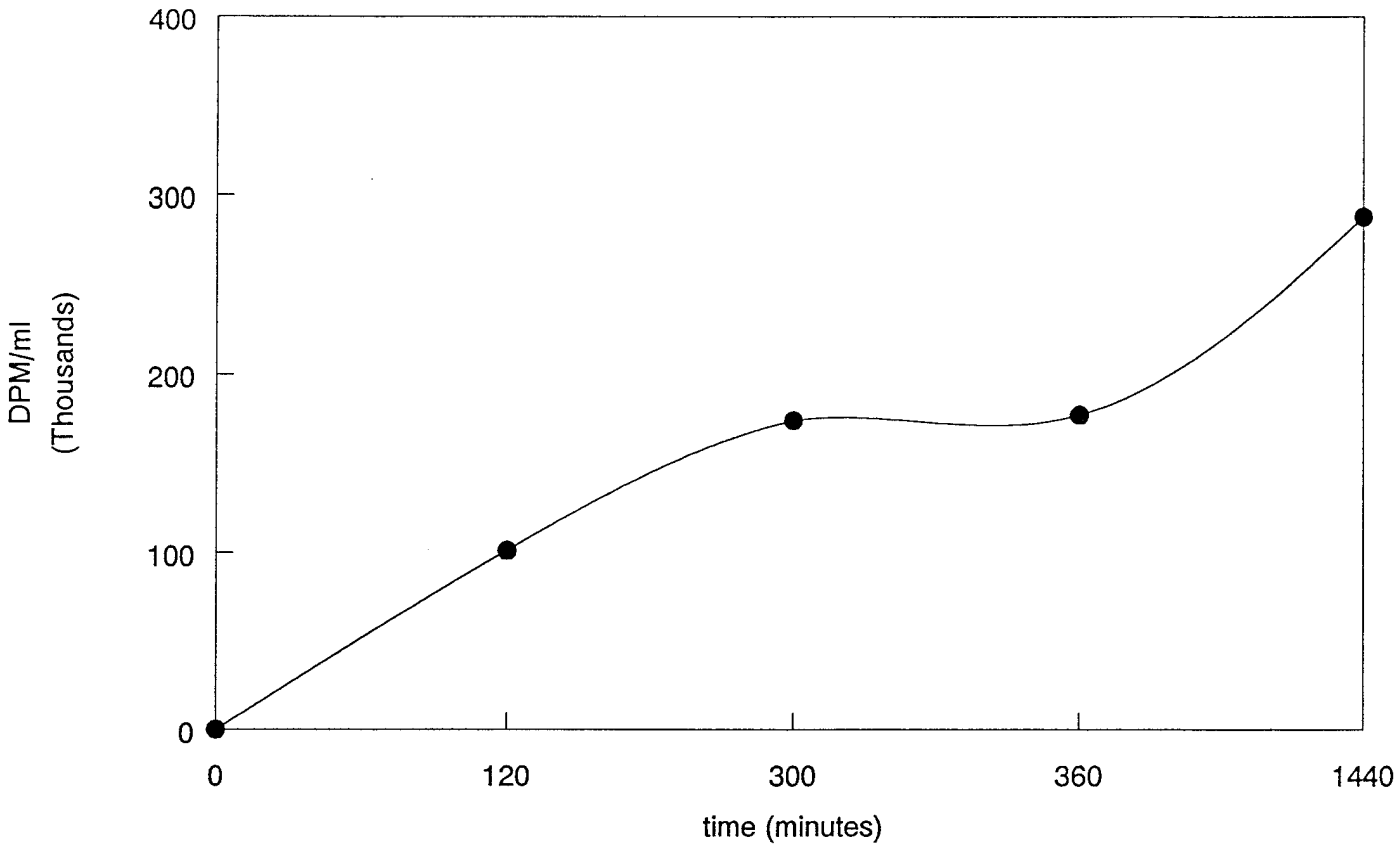

Figure 4. DPM/ml Levels of TMPP Found in Urine Post i.p. Injection

DBNP:

Efforts to isolate the metabolites of DBNP continued. DBNP urine was taken and treated with charcoal, eluted from a small C_{18} column with water, and lyophilised. The resulting crystal was reconstituted in a minimal volume of water and will be further purified via HPLC.

A crystal was found to form from the elutant of DBNP urine from a C_{18} column that had been allowed to slowly evaporate to dryness. This crystal was further purified and dried. Attempts to identify the crystal have shown that it has a melting point of 132-136°C, does not give off any

effervescence when treated with bicarbonate, and does not give off an ammonia odor when alkalinized and heated. Further analysis will be necessary to determine what the crystal is.

Cell Model:

A program was developed for culturing WB 344 liver cells. The cells are maintained in a 38°C and 5% CO_2 environment. Cells are stored cryogenically for future use. The growth rate of liver cells in this environment was measured. The number of viable cells was plotted against time.

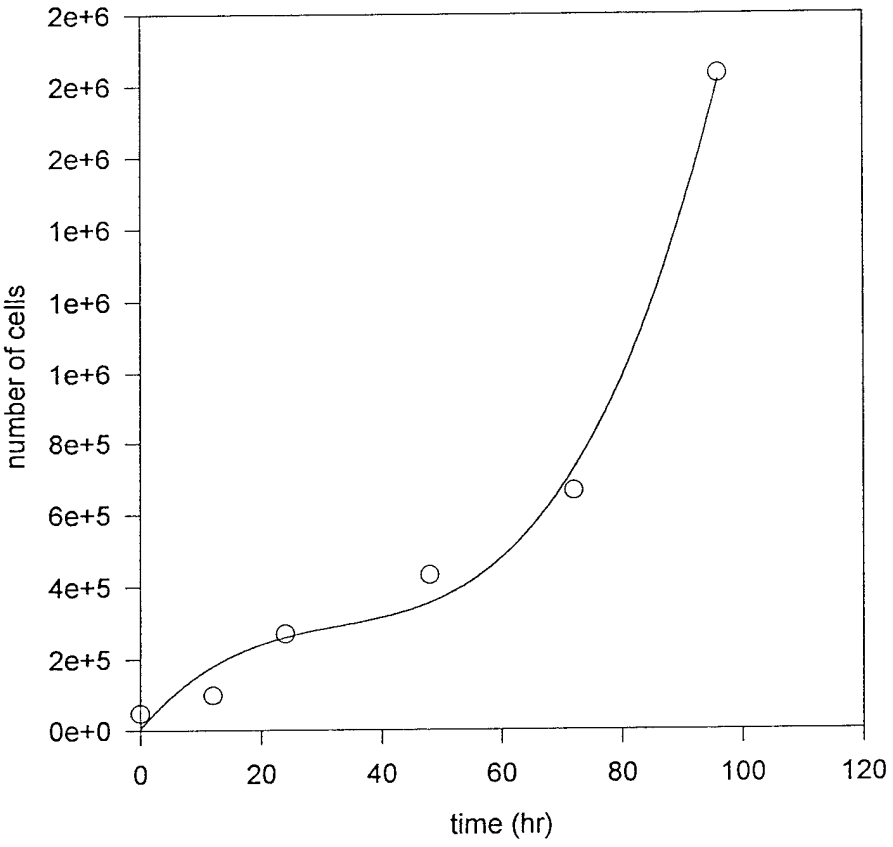

Figure 5. Growth Curve for Liver Cells

cell count at t_0=47,750 cells; cell count at t_{96}=1,832,500 cells
$\log_2(t_0)$=15.543; $\log_2(t_{96})$=20.805; # of doublings at 96 hr=5.262
Doubling time=96/5.262=18.24 hr

In the future, this system will be used to analyze the effect of toxicants on the body in place of using the entire animal.

Neurotransmitter Study:

Solutions of neurotransmitters were prepared from the brains of Wistar rats to establish a baseline for comparison to samples. The solutions were prepared by decapitating the rat and freezing the brain in liquid nitrogen. The frozen brain was then weighed and allowed to thaw in 1 ml 0.17M $HC10_4$ per 100 mg tissue. The tissue was homogenized and centrifuged at 20,000 rpm for 20 minutes. Analysis of the content of the supernatants was done by Lt. Lindsey and Sue Prues.

We also prepared several sets of samples that belonged to CMDR Rossi for neurotransmitter analysis.

Ritchie

Dr. Ritchie is a psychologist/senior scientist who conducts neurobehavioral research for the NMRI TOX/DET. His accomplishments are listed as follows:

A major new Work Unit is being written ($626 K over four years) to change the research emphasis from inhalation exposures to pharmaceutical challenge for NTAB validation. Test battery development and validation will occur by comparison of human studies reporting neurobehavioral consequences of pharmaceutical challenge with animal results on similar tests. Drugs to be compared will include amphetamine, ethanol, LSD, and a number of other drugs known to agonize or antagonize major neurotransmitter systems. Rat and pigeons responses will be investigated on a number of neurobehavioral tests before, during and following pharmaceutical challenge. This major change in research emphasis will remove the dependence upon integrity of the inhalation exposure chambers in Room 202, and will allow test battery validation to proceed in Room 205.

NTAB Development:

- Boxes for play analysis of juvenile rats were built; computer software for scoring is being developed by Bill Binole.
- Work on development of the Porsolt Forced Swim Test is progressing.
- Progressive Ratio testing of rats will begin as soon as the chambers are certified safe for exposures.

Ozone Depleting Substance Replacements:

All research efforts were terminated on 15 October 1994 by the "Inhalation Issues Committee" (Robert Carpenter, Chairperson). The Committee determined that the Hinners-type Chamber door required for inhalation toxicology testing was unsafe for use without modification. The door is now replaced, and the Committee has now determined that testing can resume on or after 17 Feb 1995. A number of animals have been shaped and pre-trained for exposures and are awaiting resumption of testing. Exposure of rats and pigeons may be indefinitely delayed if the Flow Analysis Pattern within the chambers is found to be unacceptable.

TMPP:

A brief study was completed, investigating the effects of low doses of TMPP on schedule-induced behavior in well trained rats. It was conclusively shown that TMPP administration at a dose level of 0.20 mg/kg severally disrupts operant behavior, while repeated doses at 0.10 mg/kg is without apparent effect. Results of this study indicate a possible new animal model for human absence epilepsy.

Ligand binding work in conjunction with Dr. T.K. Narayanan indicated that the novel neuroconvulsant does not bind appreciably with any of the common CNS receptors, including the GABA A or B receptors. This result is significant because it conflicts with the previously accepted mechanism of action of the drug. It was found that TMPP serves as an agonizes the binding of bicuculline, a potent GABAA antagonist.

Microdialysis work in conjunction with Dr. James Lindsey was begun, with four animals successfully implanted with microdialysis cannulas. Surgical procedures were validated, and initial dopamine scans indicates successful implantation of cannulas in the nucleus accumbens.

Gulf Warfare Syndrome:

Preliminary meetings with the Army Toxicology staff resulted in selection of subjects, exposure equipment, exposure conditions, and post-exposure tests.

Appropriate exposure cages were selected and modified in conjunction with Dr. Ed Kimmel and Bill Sontagg.

Shockers/harnesses for stress simulation were selected and ordered in conjunction with Ms. Mary Jane Walsh.

Repair and set-up neurobehavioral equipment required by the project was begun in conjunction with Mr. Bill Binole.

Martin

Ms. Martin's role in technical editing has resulted in the following accomplishments: She has helped many manuscripts through the publication and procurement processes. In addition, she has quickly and accurately recreated, transferred to slide film, and expedited the development of six briefings to be given at the Navy Environmental Health Center's conference by the Officer-in-Charge of NMRI/TD. She is also preparing a poster presentation for this conference. An additional accomplishment of this quarter is Ms. Martin's input into the storyboarding process of a Tri-Service Toxicology video to be premiered at this year's April Conference.

Binole, Rix

The computer programming and local area network expertise is provided by GEO-CENTERS and the accomplishments for this period includes:

Expansion of internet presence with upgrades to our WWW server, and the addition of gopher and WAIS servers. Additionally a list server was enabled as an experiment. If the response to this is good the software will be purchased and other lists may be setup.

Completed conversion of our main server to the NTAS NOS. An additional server was also added to act as a Lab LAN server for the storage of scientific information.

Remodeling of ADP. Although not a stated objective, it became apparent that in order to provide necessary space for documentation and to consolidate the location of all servers to the ADP area that a remodeling job was needed. This included the removal of all outdated documentation, removal of all documentation from the computer room to ADP proper, rearrangement of/painting of computer room, and the relocation of the Library information system to the computer room.

Addition of modem pooling software to the NTAS servers. This will allow all to access a modem in a logical manner in the same manner that disks are now accessed on the LAN. Full functionality of this is dependent on the rerouting of all data phone lines to the computer room.

Setup of RAS service. This is an NTAS service that allows for remote dial in to the NMRI/TD LAN system.

Development of next generation of pigeon training programs.

Begin development of a suite of test software for the inhalation group.

Collection of site specific documentation with regards to software inventory, and workstation specific information.

Ademujohn

Ms. Ademujohn provides technical support to Dr. Ritchie in the neurobehavioral research area at the NMRI TOX/DET and her accomplishments include:

- Compiled, organized, cataloged, via computer-aided graphics, the tri-weekly data on CO (500, 1000, 2000, 3000 rpms.).
- Catalogued and analyzed incoming tri-weekly data on Halon 1211 (0.5%, 1.0%, 2.0%, 4.0%) graphed data from the response curves.
- Catalogued and compiled all incoming tri-weekly data on CO (500, 1000, 2000, 3000 ppm), respectively.
- Drafted an S.O.P. (Standard Operating Procedure) for safety features and precautions to be implemented during Carbon Monoxide testing.
- Implemented an S.O.P. on the procedures for pigeon "Match" protocol training.
- Maintenance of all laboratory workunit notebooks.
- Implemented several data methods to compile training data and weight maintenance on the pigeon operants.
- Responsible for procurement of gases used for testing.
- Completed graphic schematics on pigeon operant apparatus.

Connolly

Ms. Connolly provided librarian and informational systems services this quarter as described below:

- 236 books cataloged and prepared for circulation.
- 104 articles obtained from local libraries.
- 8 interlibrary loans obtained.
- 12 books obtained from local libraries to fulfill requests.
- 72 article requests filled from this library's collection.
- 6 literature searches conducted using in-house CDROM database capabilities.

- 3 new people oriented to the library.
- 8 SOPs written covering various aspects of library procedures.
- Became a member of the base level Improvement Process Team (IPT) on Libraries and Information Centers.
- Unpacked the cartons of library books that were packed after the flood onto the newly installed metal shelves.
- Sorted through existing reprint files removing duplicates, and copier reproductions of journal articles that are duplicates of journal articles on the shelves. Removed over 300 such articles.

Walsh

Ms. Walsh supports the Armstrong Toxicology laboratory by providing the following during this quarter:

Inhalation Toxicity of Tricresyl Phosphate Vapor Phase Lubricant:

I was assigned as project leader for the continuation of this existing project. After acceptance of an addendum to the existing protocol, I accomplished testing of two tricresyl phosphate compounds, one being a synthetic and one a natural, which had been previously tested. Following reengineering of the test equipment supplied by the Wright Laboratory, Aero Propulsion and Power Directorate, Fuels and Lubrication Division, it was necessary to validate this engineering by verification of repeated Neurotoxic Esterase Assay test data. I was responsible for the coordination with the customer, organization and test apparatus set-up, ordering animals, performance of the nose only exposure, and oral gavaging for this project. A Technical Report of the test results is being prepared for submission to the customer.

Combustion Toxicity of Advanced Composite Materials:

B2 Combustion was completed following 12 burns utilizing a 70 ft tunnel connected to the UPITT II combustion module used in previous burns. Technical support was provided for all aspects of the data collection to include: pump calibration and set-up, preparation and analysis of impactors, running the computer which monitored the temperature of the combustion tunnel and monitoring combustion of the material. The data samples collected were submitted for Electron Microscopy evaluation and image analysis.

In Vitro-In vivo Extrapolation:

To date I have organized and performed nine liver profusions for primary hepatocyte isolation used to develop various assays to include MTT, LDH/AST, and Protein. This data will be used as a cellular toxicity factor which accounts for the probability that given a particular cellular concentration of the active form of the toxicant a particular cellular response will occur.

Dong

Dr. Dong conducts surgical services to the Armstrong Toxicology laboratory dermatology and skin absorption programs. Her accomplishments include:

- Obtained the gas uptake and distribution kinetic (k_m, V_{max}, and K_{fc}) data of perfluorohexyl iodide in F-344 rats by performing closed chamber gas uptake experiments and using a PB-PK model to describe mathematically the disposition and metabolism of the chemical.
- Measured CPFB absorption in Hartley guinea pigs which resulted from dermal exposure of CPFB.
- Accomplished a poster for presentation in upcoming 1995 SOT annual meeting.
- Accomplished the a technical report titled "Dermal absorption kinetics of liquid chloropentafluorobenzene (CPFB) and 1,2- dichlorobenzene (DCB) in rats and guinea pigs".

Grabau

Dr. Grabau has provided pathology and image analysis expertise to the US Army and US Air Force toxicology programs. His accomplishments include:

Species Differences in Skin Penetration:

- This investigator participated in meetings (in-house and off-site) to support the new dermal research group effort determining the feasibility of selected dermal research initiatives, which are being evaluated for potential AFOSR funding. As a task of image analysis support, a survey of available research immuno-histochemical reagents to quantifying early events in chemically-induced skin inflammation was conducted. Two candidate reagents have been selected and ordered but have not arrived.
- An abstract (first author) for poster display was accepted for presentation at the 34th Annual Meeting of the Society of Toxicology. It will detail image analysis methods for evaluating epidermal thickness in dermal toxicology studies.

- An abstract (first author) for poster display was accepted for presentation at the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995). It will detail an image analysis algorithm used for comparison of dermal vasculature characteristics in dermal toxicology research.
- Dermal histopathologic diagnosis and interpretation has been provided to support this research effort to develop a PB-PK model for dermal exposure of toxic chemicals. These research findings will also be included in a poster presented by Dr. L. Dong (first author, I am a co-author) at the 34th Annual Meeting of the Society of Toxicology. It is anticipated that these research findings will also be presented at the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995).

Combustion Toxicity of Advanced Composite Materials (ACM):

Accomplishments for this period include conducting image analysis of over 700 images obtained from twenty one separate combustion experiments of ACM in a modified UPITT II combustion chamber. Each image often had 200 to 800 features (ie particles) which were evaluated by 16 different parameters. Abstracts (second author) have been accepted describing the methods and results of this research program at the 34th Annual Meeting of the Society of Toxicology (March 1995)and the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995).

Trichloroethylene (TCE) Biologically Based Health Risk Model:

Image analysis methodologies were developed and conducted to quantify subcellular (PCNA and P450 isoenzyme) changes in liver cells exposed to TCE. The results of this research will be presented as posters (second author with Mr. K Geiss and coauthor with others) at the Society of Toxicology Annual Meeting (March 1995). It is anticipated that these research findings will also be presented at the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995).

Intra-Laboratory Research (ILR)Grant - Hepatic Apoptosis in Mice and Rats:

This researcher participated in planning and scheduling meetings to allow accomplishment of research goals. Specifically, image analysis will be conducted on tissues collected from 100 rats and 200 mice to study apoptosis.

Other Tasks:

- CRDA - I am currently the point of contact and coordinator for preliminary efforts to establish a cooperative research development agreement (CRDA) associated with computer-based image archiving and telecommunication of digital images.

- Consultation for Tri-Service Marketing - I am currently serving as a consultant to the Tri-Service Marketing Group regarding implementation of multimedia and audio-visual objectives.

- Coordination of Image/Presentation Resources - I serve as a focal-point for computer-based graphic aids, research-associated photographs, and other presentation aids. I evaluate and provide recommendations for hardware and software for these areas.

- Consultation for Laboratory Computer Requirements - I am currently serving at the pathology group liaison to the CETA computer consultants. This has involved consultations regarding pathology requirements, GLP requirements, statistical software requirements and computer-based image/graphics requirements.

Geiss

Mr. Geiss provides technical expertise to the Armstrong Toxicology Laboratory and has accomplished the following:

- Hybridization assays have been performed on samples from the 60-day study with probes made from plasmid templates for ICAM-1, GCS-LC, and GCS-HC. Other templates have been prepared for TGF-b1, PKC-a, ODC, and IL-1a.

- For method development and experimental design, time was spent in literature searching and computer analysis of nucleic acids. Literature searches were necessary to determine what hybridization parameters were needed and what results were to be expected to confirm our analysis of specific mRNA targets. Computer analysis of the nucleic acid sequences was used for determining restriction fragment sizes used in confirming probe identity.

- A significant portion of my time has been spent in developing in situ hybridization assays for analysis of mRNA in paraffin-embedded tissue sections. The method for histone mRNA analysis is now ready for use in the liver tissues. The analysis is assisted by an

automated slide stainer. This instrument allows for a large number of samples to be processed at one time and adds greater reproducibility between samples. The current probe I am using is for histone mRNA. This probe is useful in detecting proliferating cells. Since the cells are actively synthesizing DNA, histones are needed to package the DNA. There is precedence for the induction of cell proliferation by TCE. We are studying whether we can detect TCE-induced cell proliferation from our 60-day study. Immunochemical analysis has shown increased PCNA protein levels, which should correspond to cell proliferation.

- Northern analysis has been accomplished for GAPDH and ICAM-1 on the RNA samples of the HBO project. Results show no statistically significant difference between the samples for the control (GAPDH) and ICAM-1 probes.

- Recently, 6-8 hours a week have been spent in training a technician in molecular biology techniques. The training is achieved in conjunction with actual experimental work.

IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND ARTIFICIAL BLOOD STUDIES

Artificial Blood Group (NMRI)

The following accomplishments for this group includes:

Studies have been conducted to establish the nature of the relationship between extended red cell storage and chloride depletion. Units of red cells have been divided into four aliquots, each of which was washed to a different extent resulting in different degrees of chloride depletion. The rate at which the morphological index decreased during the subsequent six weeks of storage appears to be roughly proportional to the extent of chloride depletion. However, it was apparent that beyond six weeks of storage, the rate of glycolysis becomes the dominating factor in determining the ultimate duration of storage. After six weeks of storage the 2,3 DPG fell precipitously, ATP began to fall more sharply and the morphological index decreased more rapidly. These factors in turn appear to be related to a fall in pH to below 6.5 at which time the consumption of glucose and the production of lactate decreased markedly. These observations imply that at least two factors are critical in extending the shelf-life of refrigerated red cells: chloride depletion to maintain good morphology and reduce hemolysis and the maintenance of elevated pH to prevent the slow down of glycolysis and loss of ATP.

The previous progress report described the indirect evidence that apoptotic leukocytes in washed cell suspensions released surface molecules which then adsorbed to red cells during storage and resulted in the phagocytosis of the red cells following transfusion. Further survival studies to confirm these observations are pending the availability of funding of a proposal specifically directed at applied studies of extended storage of washed red cells. In the meantime, based on our preliminary observations, we have attempted to demonstrate the transfer of phagocytosis signals to red cells during storage. A phagocytosis assay was developed in an effort to observe the phagocytosis of red cells *in vitro* following the addition of fresh granulocytes to a sample of stored, non-filtered, washed red cells. This was a difficult assay to design and proved to have insufficient sensitivity to detect the degree of phagocytosis implied by the clinical data.

Since two signals responsible for the phagocytosis *in vivo* of apoptotic cells have been identified, namely thrombospondin is commercially available and an antibody conjugated to a fluorescent tag for use in the flow analyzer was obtained. We have not been able to demonstrate thrombospondin on red cell surfaces by this technique. Antibody against phosphotidyl serine is not commercially available but has been developed by Dr. Douglas Green of the La Jolla Institute of Allergy and Immunology. He has agreed to provide us with this antibody. At the moment it has yet to be directly demonstrated that the loss of stored red cells following transfusion results from the acquisition of a phagocytosis signal. The relationship between pre-storage filtration and improved 24-hr survival need to be confirmed by additional survival studies. While waiting for antibody from Dr. Green, we will be exploring alternate approaches to be phosphotidyl serine assay.

Li

Dr. Li conducts wound repair research for NMRI and has accomplished the following:

- We have worked on HS model continuously, anesthetized the mice with halothane and bled through the cannula in the femoral artery. Fed with cytokines (CAS, IL-6, TNF, IL-2) or control media after HS.

- Measuring the number of bacteria in liver and determining the spleen cell proliferation stimulated by Con A after HS.
- Investigated the pathological responses of intestine , and prepared the mice intestine samples for histological study.

- Hepatocytes cell lines (BNL, BNLMea, HER G2), intestine epithelia cell lines (HCT-8, HTB-40) and endothelioma cell line (EOMA cells) have been stimulated with various concentrations of LPS, incubating in 1% oxygen, 5% carbon dioxide and 94% nitrogen at 37°C.

Above cells were incubated on chamber slides fixed with 2% glutaraldehyde, 1% paraformaldehyde in 0.1M sod. cacodylate buffer using HE staining to examine the cell morphological damages induced by LPS and low oxygen conditions mentioned above.

Using Neutral Red assay (NR assay) to measure the cellular deteriorations induced by LPS and low oxygen .

- Oral administered cytokine can be of benefit in the prevention of bacterial colonization of peripheral tissue following HS. Oral IL-6 improves gut barrier function and reduces bacterial translocation to the periphery, partially restores peripheral immune function after HS.

- According to the results of NR assay and HE staining, the cellular deteriorations following endotoxic and hypoxic insults can be induced by the minimal concentration of LPS (0.5 mg/ml).

Fan

Ms. Fan conducts molecular biology in support of NMRI and has accomplished the following:

- Generated satisfactory results on nitric oxide synthase mRNA expression in rat cardiac myocytes and aorta smooth muscle cells using RT-PCR and Southern blot. The cells were treated with LPS, beta-phorbol (PKC activator), L-NAME (nitric oxide inhibitor) and sphingosine (PKC inhibitor) appropriately.

- Completed PCR primer and probe selection for PKC. The synthesized oligonucleotides have been successfully tested.

- Set up the non-isotopic DNA sequencing system and a known DNA has been used to test the procedure successfully.

- Completed the planned project in colaboration with Dr. Volgo.

Chavez

Dr. Chavez provides blood research for the joint program between NMRI and WRAIR. The accomplishments for this period include:

Three production runs were completed during this quarterly period. The first production run of chemically-modified hemoglobin at this location was accomplished the week of 14 November 1994. The primary goal was to evaluate the equipment and standard operation procedures in place. Mechanical problems with the bioreactor were identified and fixed; a nitrogen gas leak allowed oxygen to enter the reaction vessel. As a result, the cross-linking efficiency was only 78% compared to typical values of 95-97%. Otherwise, the production run was quite successful for an initial run. The next production run was performed in December to familiarize Bionetics personnel with the pilot plant operations. With the assay methods in place, I assisted in training personnel in both operation of the equipment and interpretation of the data.

The standard operation procedures were evaluated in January 1995 and changes were incorporated for the 6 February run. I recommended and modified the red blood cell washing to remove plasma. By reducing total volume requirements, about one hour of production time and ~200 l of sterile saline solution was saved. With similar changes to the lysing procedure, another hour of production time and ~200 l of sterile buffer solution was recovered. For the cross-linking of the hemoglobin, the use of HEPES buffers was eliminated because it is not approved in the U.S.P. guidelines and is also expensive. Preliminary results indicate that the cross-linking reaction is more efficient, with the desired product raised from a previous high of 44% to 54% of the total product. Subsequent runs will validate this result. Overall, the pilot plant is in full operation and working well. Modification of the procedures will be an ongoing process in order to optimize the production.

The heme affinity experiments have been very successful. Complementary heme exchange experiments have revealed that cross-linked hemoglobin derivatives lose their hemes at a slower rate. Our results have shown that the heme affinity for the cross-linked hemoglobin is equivalent to native hemoglobin. However, the cross-linked hemoglobin incorporated the heme at a slower rate than native hemoglobin. By utilizing singular value decomposition on the absorption spectra, we have determined that protein folding is the controlling factor in both heme loss and heme association. In order for the heme to be released or bound to hemoglobin, the protein must undergo some degree of protein folding/unfolding. By cross-linking the hemoglobin, the motion of the protein is restricted. Thus, the folding/unfolding rate is retarded. This explains why the kinetic rates for the cross-linked hemoglobin are slower for both heme loss and heme association. Subsequent studies will look at mutant hemoglobin and other chemically modified hemoglobins.

I submitted and presented this data at the 39th Annual Meeting of the Biophysical Society in San Francisco, CA.

Dr. James Manning of The Rockefeller University was in the laboratory on 27 January 1995 to do collaborating experiments on the rapid scanning spectrophotometer. Dr. Manning specializes in modifying hemoglobin through site-directed mutagenesis and also chemical cross-linkers. A carbon monoxide - oxygen exchange experiment was performed with Dr. Manning's mutant hemoglobin. This particular hemoglobin exists primarily as a dimeric protein instead of the normal tetrameric form. Interestingly, although the mutant hemoglobin is a dimer, it exhibits similar properties to native hemoglobin. Ongoing collaborating experiments to compare this mutant hemoglobin and native hemoglobin should reveal the contributions of specific amino acids to the function of hemoglobin.

Ring

Dr. Ring supports the bone marrow research program at NMRI and has accomplished the computer science during this period:

A complex database program was written in Foxpro (a dBase competitor) to track mice breeding, experiments, and bloodlines. Hundreds of mice are used in experiments. The previous collection of spreadsheets was becoming difficult to manage as the data volume increased.

Assistance was provided in spreadsheets and databases for use by I.C.B.P.'s administrative personnel.

More investigators were brought "online" to the network. Training continued in the areas of E-mail and file sharing.

Services were provided to run the calcium imaging system.

DENTAL DISEASES-RELATED RESEARCH

Zablen

Dr. Zablen performs molecular biology expertise to the NDRI. His accomplishments for this period includes:

SINGLE CHAIN FRAGMENT VARIABLE (ScFv) RECOMBINANT ANTIBODIES:

- Initiated production of recombinant Mab cloning/expression in E. coli. Mab BG II, F9/2D immunized with P. gingivalis ATCC 33277 was used to produce polyA mRNA for cDNA synthesis and PCR amplification. A Linker oligo was attached and PCR performed to amplify a 750 bp ScFv fragment ready for cloning into phagemid pCANTAB 5E. The ScFv fragment was successfully cloned into the phagemid and potential clones were screened for production of soluble antibody. Several clones were identified by ELISA plate assay. Expression studies were performed and analyzed by western blot. Clone E9 appears to be the best candidate for further study. Clone E9 was prepared and the ScFv found to accumulate in the periplasm of E. coli after induction. ELISA tests confirm the biological activity of the antibody in this subcellular fraction. The E9 clone was preserved as a lyophilized culture and as a plasmid prep for long term storage. These reagents were turned over to the funding sponsor.

Turner

Dr. Turner also provides senior scientific expertise to NDRI and has provided the following:

We have received permission from our parent Command to purchase fluorescent spectrophotometers for measurement of neutrophil biological activities. This equipment will increase the sensitivity of our assays up to ten-fold. Government procedure has required that purchase of these items be placed on "bids" from potential vendors. The bidding process is now complete and the purchase orders have been placed. It is estimated that the equipment should be in place and functional by April 1, 1995. In the meantime, crude assays on neutrophils derived from healthy subjects have been analyzed using the SMART System and profiles of potentially interesting lysosomal enzymes have been developed on the System.

Two abstracts related to the work have been accepted for presentation to the American Association for Dental Researchers (AADR). The papers will be presented at the annual AADR meeting in San Antonio, Texas during the week of March 6, 1995.

Spencer

Ms. Spencer provides technical services to the NDRI clinical research and has provided the following during this reporting period:

- Completed literature review of alveolar osteitis (AO) and statistics review.
- Completed a steroid contraceptive identification and review guidebook.
- Coordinated AO study sites at three dental clinics (bldg 1017, bldg 237 and the hospital).
- Processed data for all patients used in the AO study (as of 2/27/95 - 165 patients).
- Trained personnel on interviewing and data collecting techniques.
- Assisted Dr. Mark Cohen in the review of steroid contraception for his publication: "Effect of Gender-Related Factors on the Incidence of Localized Alveolar Osteitis."
- Assisted in the training of enlisted personnel for the promotion exam.

Lamberts

Dr. Lamberts has conducted the following technical editing of the NDRI manuscripts:

- Edited four manuscripts (Simicek [2], Deutsch, Meyer) to be submitted for publication.
- Extensively revised a "response letter" from a correspondent of Dr. Simonson for the Editor of the Journal of Periodontology.
- Reviewed five posters [Schade, Pederson, Turner, Deutsch, Leal] to be presented at the March AADR meeting in San Antonio, Texas.
- Completed a manuscript on "Salivary levels of alpha-2 macroglobulin, alpha-l antitrypsin, C-reactive protein, and polymorphonuclear neutrophil cathepsin-G and elastase in human subjects with or without destructive periodontal disease". The manuscript has tentatively been accepted for publication by the Archives of Oral Biology, pending modifications according to the reviewers' suggestions. Work on these modifications is in progress.
- Began preparation of a manuscript on "Quantitative Relationships of Eikenella corrodens, Porphyromonas gingivalis, Treponema denticola, and Wolinella recta to the Severity of Localized Juvenile Periodontitis" from data of Dr. Simonson.
- Reviewed three Masters theses of Northwestern University dental students for the possible preparation of a manuscript. The theses had been written from studies conducted under a collaborative arrangement between Northwestern U. and NDRI.
- Edited four research manuscripts, as well as a response letter, that were to be submitted for publication.

- Reviewed five research posters that are to be presented at the March, 1995 AADR meeting.
- Completed and submitted for publication a manuscript on salivary levels of various immune-response indicators in relation to periodontal disease status. The manuscript has tentatively been accepted for publication.

Ahlf

Ms. Ahlf has performed the following NDRI clinical research functions:

During this first quarter I have had the distinct pleasure of meeting strategic individuals that will facilitate action regarding our research efforts in the Naval Dental Clinics. These people are either Clinic Directors, paving the way for us to come into their clinics with little adversity, or Clinical Personnel who will be participating in some way with the research projects.

In the process of establishing relationships with the above people, a request was submitted to research the availability of a 2-3 minute video tape on smokeless tobacco. The hygienists at 1523 were wanting to include this tape with their oral hygiene classes. Out of that request, a point paper has been drafted with the intention of exploring the possibility of developing and evaluating a tobacco cessation program designed specifically for the Naval recruits.

Traveled to Kansas City to the Central Records, Manpower and Management, Personnel and Management Division, Marine Corps Reserve Support Command for the purposes of collecting dental records. This mission was to support the research project, Emergent Dental Occurrences in a Military Setting, A Retrospective Study of Dental Emergency Visits During Operations Desert Shield/Storm.

The Credentialing Committee has granted me clinical privileges provide non-surgical preventive services at the Naval Dental Clinics.

Began working on a project prompted by the Bureau of Medicine and Surgery. The issue is dental examination periodicity in the Navy Dental Corps. The next step is to explore further with the assistance of Dental Officers the necessity of yearly T2 examinations. A specific time line has been established.

Miller

Dr. Miller has accomplished the following science in support of the NDRI Detachment in Bethesda:

Relative to the project Evaluation of the Influence of Superantigens and Polyclonal B-cell Activators in Periodontal Disease we have completed the writing of a manuscript titled ╥Immunomodulation by components of bacteria associated with periodontitis. This paper completes the first section of the IR. and deals with the possibility of superantigens in bacterial preparations as well with the nature of the immunosuppressive factors associated with the bacteria. In addition, and something that is considered cutting edge research and technology, we have utilized quantitative reverse transcriptase PCR procedure to identify superantigen production in bacterial preparations by the identification of specific Vb message production by lymphocytes exposed to bacterial superantigens. This procedure incorporates the use of a state-of-the art fluorescence gene sequencing procedure and involves a collaboration between my laboratory and the National Institutes of Health Epidemiology and Disease Prevention Branch of NIDR (Dr. Scott Diehl). In addition, we have also run a number of studies to evaluate the expansion of T-cells producing various TCR- Vb markers on cells exposed to bacterial components using two and three parameter flow cytometry. Two abstracts have been written relative to this program and have been accepted for presentation at the American Association of Dental Research Annual Meeting and the 9th International Convocation of Immunology.

Considerable work has also been completed concerning the influence of Treponema denticola on cytokine production by cells obtained from periodontally diseased and non-diseased individuals. A minimum of 6 control and 6 periodontally diseased individuals were tested and responses to T. denticola appear to be elevated in the diseased group.

Relative to freeze storage of lymphocytes we have evaluated IL-1, IL-2, IL6, IL-6sR, and TNF production by lymphocytes maintained in liquid nitrogen for extended periods of time. An abstract has been submitted and accepted for presentation at the American Association for Dental Research Annual Meeting.

Relative to the project "Clinical evaluation of bacterial leakage of endodontic temporary filling materials", we have written the final manuscript and it has been submitted for publication.

Relative to the project "Influence of growth factors on gingival and periodontal ligament fibroblasts."

We have completed much of the planned research and anticipate the completion of a manuscript by late spring. I have also begun negotiations with individuals at the Portsmouth Naval Hospital to utilize their research facilities to continue this work under the supervision of Dr. T. Barco who is now stationed there.

Two new IR proposals have been written and submitted under the name of LtCdr M. d'Alesandro for funding in the fall of 1995. Preliminary information indicated that funding for one of these programs will be made available.

The Dental Immunology Course has been completed and grades submitted.

Gu

Ms. Gu works with Dr. Miller and has provided the following technical functions for his research:

In order to obtain a quantitative PCR product and reduce an artificial PCR product, several conditions have been tested using eight samples and three individual RNA preparations. This initial focus has included:

- isolation of total RNA by using a tissue culture isolation kit;
- establishment of reverse transcriptase reaction condition;
- establishment of cDNA concentration for a 20 ul PCR reaction;
- determination of proper PCR cycle numbers;
- determination of the most effective PCR temperature;
- the utilization of twenty two Vb primers in testing;
- the establishment of an internal control system;
- PCR products dilution for a denature gel;
- establishment of the most effective PAGE gel size, and;
- an understanding of the limitations on software.

According to these previous testing results, a RT-PCR technology system for identification of T-lymphocyte Vb mRNA was developed. For each individual sample, about 2 x 106 T-lymphocyte cells were used as raw material for preparation of total RNA and first strand cDNA. These cDNA will be enough to serve for two replicates of the assay utilizing all twenty-two Vb primers. In order to standardize the assay and start a equal PCRU able cDNA concentration in a 20 ul of PCR amplification, each first strand cDNA was equalized to achieve to a 500 peak high level (determined by Applied Biosystems) before the PCR reaction. Each PCR cycle was run at three different temperatures, 95°C (denature), 60°C (annealing), 72°C (elongation) for 1 minuet

each. However, after 20 cycles, 10 ul was removed from the reaction mixture and the remainder (10 ul) was incubated for 5 additional cycles. Finally, each sample was tested with twenty two Vb primers and two cycling system, respectively.

Forty-nine different T-lymphocyte cultures pretreated with different superantigen and bacterial stimulants were used for determination of Vb mRNA level. In the recent quarter, all the RNA isolation were completed as well as the first string cDNA synthesis, and cDNA concentration titration.

Relate to oral cancer project:

- genomic DNA was isolated from seven culture cell lines (the samples were prepared by Dr. Schwartz); and,

- DNA expression was determined with the aid of a spectrophotometer and agarose gel with ethidium bromide standing.

Solanki

As with Ms. Gu, Ms. Solanki provided Dr. Miller and the NDRI Detachment in Bethesda with the following technical support:

We have successfully concluded the freezing study experiments and now are in process of measuring various interlukin/cytokines activities using commercial ELISA procedures. In this regard, various supernatant from cells cultured with antigens and superantigens are being tested for IL-2, IL-1B, IL-6, and TNF-B.

Lymphocytes of many subjects have been setup for "Influence of T. Denticola on cell proliferations" experiments. Their supernatant have been frozen for future studies to detect the levels of IL-2, IL-1B, IL-6, and TNF-B.

Lymphocytes from two sources have been stimulated with ten different superantigens to evaluate and compare the messages of nucleic acids among each simulators and subjects at molecular level using a sophisticated procedure called polymerase chain reaction (PCR).

We are making progress in separating proteins of crude bacterial prep by continuously modifying and introducing new ways to make the separation process more accurate and reproducible.

BIOMEDICAL DIVING RESEARCH

Shea

Dr. Shea, supporting the NMRI diving medicine department, has accomplished the following:

Experiments were conducted to verify that injections of either lidocaine or TTX would reduce the extracellular levels of ACh in the cortex. The results of this work indicated a twenty to forty percent decrease which are the expected values for this nerve pathway. The injection of 50mM NMDA however, has shown to increase the levels of ACh too at least 100% of pre-injection values. This has been maintained for up to five hours after injection. The data however are from only a few animals thus far. Probe and injection sites were verified and adjusted by the examination of brain tissue slices. The contralateral side has only received a microdialysis probe and the results thus far show little if any effect on ACh levels.

Statistics for the cold study were completed. Significant differences were noted between animals at room temperature verses cold for levels of ACh in the frontal cortex. Animals exposed to cold had higher levels then when compared to their baseline values. Only a few time points were different between animals exposed to cold and those exposed while receiving either 5 or 10mM glucose. The ACh levels in general were lower in the glucose animals over time as compared to saline animals.

Two companies have been selected for possible instruments that will meet our needs for *in vivo* nitric oxide measurements. They are both based on voltametry methods and a final decision will not be made until we directly test these in our labs.

Kerr

Mr. Kerr works with Dr. Shea and has provided the following technical support:

ALZHEIMER STUDY - During the report period stated, 10 animals were lesioned with no success in depletion of ACH for five hours of post-lesion collections. Another 10 animals were used to determine the reason for the lack of success. For these animals, I infused lidocaine (20% and 2%) and TTX (tetratodotoxin) into the NBM to simulate a lesioning affect. Both these drugs, if properly infused, give a 30 to 50 percent decrease in ACH. Using these decreases as a gauge of depletion, I made adjustment to the parameters:

- adjustmented the probe placement (verified through histological techniques).

- adjustmented the infusion site (verified through histological techniques).
- adjustmented the concentration of cholinesterase inhibitor.
- change the probe lengths.
- change the vehicle of the drug.

We also made additions to the protocol by adding a second probe; going to a bilateral system. The advantage of a bilateral system allows the other side of the brain to be used as a control, if there is no 'cross-talk' occurring. The percent of decrease between the two sides is an indication of the degree or extent of lesioning to the NBM.

DIVING PHYSIOLOGY - Modification of dialysis equipment to engineer's specification and the building of an air-tight animal cage has been accomplished. All the equipment is ready for the chamber and ready to run, but as of the first week in February, chamber modifications were put on hold for an undetermined period of time. Reasons for delay due to space availability and reassigning of lab space for oxygen work units.

Porter

Mr. Porter provides the diving medicine program at NMRI with engineering and technical support to the testing chambers and to the soda line quantitative analysis program. His accomplishments are as follows:

- One lot of soda lime was received from the manufacturer and was tested for contamination.
- Short term testing program has been completed for two candidate C02 analyzers, and they are in the long term test phase.
- Short term test are now being conducted on two other candidate analyzers.
- Short term test are scheduled to begin the week of March 1 on fifth candidate analyzer.
- Hyperbaric testing of two candidate CO2 analyzers for dry deck shelter use is under way.
- Performed other laboratory as requested.

Ruby

Mr. Armand Ruby provides analytical chemistry support to the NMRI diving medicine department. His accomplishments include:

An extensive literature search has been completed on portable carbon dioxide analyzers for both the NSMRL and NAVSEA projects. Four candidate analyzers have been purchased and placed

on long term test for the NSMRL project. Upon completion of testing, one candidate and one alternate will be recommended to NSMRL for final selection.

Two candidates have been selected for the NAVSEA project. Modifications have been made to both to permit their operation in hyperbaric chambers at 6 atmospheres absolute (ATA). One candidate is presently on track and pre-prototype units have been ordered from the manufacturer that incorporate the design changes required for hyperbaric use.

As part of this program, gas samples from the divers air banks on all of the host ships will be completely analyzed to insure that there is no interference from the divers breathing air with the analyzers under hyperbaric conditions. Six subs have been sampled to date at Groton, CT, Norfolk, VA, and Pearl Harbor, HI.

Support has been provided, as required, to other functional areas of the diving medicine department.

PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

Salander

Ms. Salander supports the NMRI thermal stress program and has provided the following during this last reporting period:

It has been demonstrated that CRF has been shown to influence a variety of biochemical, physiological and behavioral processes which are thought to reflect the cascade of internal events activated during acute stress. CRF causes a substantial increase in the release of central catecholamines, especially norepinephrine (NE). It is believed that CDAP will attenuate the behavioral stress effects caused by CRF. WE have added blood studies to the design of this experiment to better understand the physiologic response experienced by the rat when CRF has been administered and the stress effects have been suppressed in the rat behavior. This study is now complete and we are currently analyzing the data.

Work on a manuscript regarding the results of the AVP/CRF study is in progress.

Wolf

Mr. Wolf conducts project managment functions for the NMRDC Combat Casualty Care program. His accomplishments include:

Continued as an active participant in the interim- report review process for all submissions from all performers. It is my responsibility to monitor, communicate with Principal Investigators, resolve problems and issues, and recommend head-quarters actions.

OCNRMIS Procurement Requests:

Thrust: Using OCNRMIS, fund ongoing contracts and initiate new contract actions by cutting Procurement Requests. OCNRMIS is the Office of Naval Research data management system for keeping track of research proposals, approvals and funding actions appurtenant thereto. It is accessed via computer and modem.

Status: Created 25 Procurement Requests; each was approved by the Program Council at ONR and the contractors have been funded. Will continue to use OCNRMIS to track the obligation and expenditures for the completion of the contractor efforts. While this evolution required a learning process ... benefits are valuable to NMRDC.

Transport Code 45A From Enable to dBASE:

Thrust: Migrate 45A data management actions from the ENABLE platform to a dBASE platform.

Status: Function and Structure decided and created. Data conversion for Enable SS files completed. Program files created. Menu drivers completed. Essentially, the APPLICATION SYSTEM is complete; minor changes will be made as Code 45A sees the need.

Animal Use Data Release Actions:

Thrust: Develop 1498 for each work unit involving animal use for sighting/review by congress per their data call and the resolution of the responsible military veterinarians.

Status: Developed report form for print out of data. Presented completed report to LTC Bley, lead veterinarian, on 27 Dec 94, 18 days prior to suspense date.

FY96 GUIDANCE Resource Allocation Record (RAR):

Thrust: Develop RAR and submit to Code 421. Must include all work units, giving pe, proj, task, wu (numbers), funds, and total).

Status: Provided figures for all work units and reviewed final product. Completed.

FY96 Laboratory Guidance Narratives:

Thrust: Prepare Narrative Guidance for work units in accordance with directives.

Status: Coordinated within code-45, second & third drafts completed. Memo from Code 45 sent guidance to Code 421.

FY96 Lab Project Report:

Thrust: Prepare the report in accordance with directive for submission to ONR and to the subordinate laboratories.

Status: Intermediate draft of 18 pages completed in 15 days. 12/20 - smooth draft to 04 for sanity check and further taken to ONR for sanity check II without negative comment. Smooth draft completed and approved within Code 45. Submitted 24 page completed document 2 days prior to suspense date.

FY96 RDDS Congressional Submission:

Thrust: Prepare the FY96 Research & Development Descriptive Summary document for final submission to Congress.

Status: Prepared and submitted document for approval within Code 45. Prepared signature sheet for senior echelons; CO, NMRDC and Mr. Montgomery at OP-911 have read and approved. Submission completed 2 days prior to suspense date.

University Affiliated Research Centers' (UARC) Management Plan:

Thrust: Identify Points Of Contact, funded UARCs and complete Resource Information form on each UARC.

Status: Drafted initial response. Gathered data for forms; categories clarified and draft Resource Information form completed. [This was one of those reports where the originator was searching for what he wished to be reported ... it took some stern negotiation to reach an agreement on what was necessary, what was superfluous.] Submitted on schedule.

Enhanced Liposome Encapsulated Hemoglobin Proposal Review:

Thrust: Read the twelve proposals and make comment. See if we agree with the paid reviewers.

V. GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD:

INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

Niu

Dr. Niu conducts enterics research for NMRI and is anticipating the following for the next quarter:

- Look for the suitable restriction enzymes which could generate the DNA fragments for complementation of mutant strain.
- Clone the DNA fragment.
- Try to identify the invasion gene(s).

Jendrek

Mr. Jendrek will support the NMRI and USAMRIID team research in the following way:

Next Quarter he will update his batch record from final draft to final copy. He will then need to perform more fermentations using this protocol for the monkey trials and for general research use. He will again need to repeat a fermentation for Toxinology of *Pichia pastoris* BOT-E. He will also have to perform a fermentation using a PA producing strain of *B. subtillus* for comparison studies. He will also be required to purify the PA produced by this strain. He will install a new centrifuge for the harvesting of the 20 liter fermentor, and create all documentation for the use of this equipment. He will also start on a batch record for the fermentation of *B. anthracis* in the 20 liter fermentor. Other objectives as yet unknown.

Weeks

The objectives for next quarter for Ms. Weeks at USAMRIID are to continue the mapping of the pFra plasmid, continue the construction of the pFra library, and to continue training the new Army technician in the laboratory.

TOXICOLOGICAL STUDIES

Briggs

Many of the activities and projects conducted this quarter will be continued next quarter at the NMRI TOX/DET. They include:

The transition will continue with the primary focus on increasing productivity and efficiency, and improving the project planning and documentation of procedures and processes. Dr. Briggs will assist with the writing of the WUISS submissions as tasked. He will participate with additional program development efforts to bring in additional funds to NMRI.

Additional toxicology studies will be conducted on the ODS refrigerant and fire suppressant during the next quarter. The most important milestones during next quarter will be to manage the metabolism studies, assist with the Pre Manufacturing Notification (PMN) submission to the EPA which will permit the Navy to begin use of the new products. By June 1, a decision relating to future safety studies will be made based on a review of all toxicology data.

The validation process for the new reproductive system risk assessment model will be initiated next quarter using several positive control chemicals. Preliminary data will be presented at the Spring Conference.

The Quality Management Program will be approved during the next quarter. Efforts to improve the quality and integrity of data produced by NMRI/TD will continue to be developed.

Efforts to create and validate new models for cardiac sensitization and both male and female reproductive system human health hazards will continue during the next quarter, and will be finalized in the Fall. These methods will be reported within six months of validation.

Bowen, Kimmel, Reboulet

The engineering team for the aerosol and inhalation research expect to accomplish the following:

Completion of the Operant/Behavioral inhalation exposures to 4% (40,000 ppm) R-134a to test ratio straining.

Pending results from 4% R-134a test, initiation of tests at the next R-134a concentration level or initiation of ratio strain tests with another test material (e.g. CFC-12, Halon 1211).

Further repair and/or modification of the NMRI/TD 250 L whole-body inhalation exposure chambers.

Assimilation of the aerosol data collected during off-site characterization studies.

Smith

Dr. Smith will accomplish the following on the Halon replacement program:

- Complete characterization studies on SFE Formulation A and write final report.
- Begin characterization studies on SFE Formulation C and D.
- Conduct range finding study on SFE Formulation A.
- Write final reports on SFE Formulation A pilot studies.
- Advance the discussion of developing a new screening procedure for cardiotoxins.

Prues

Ms. Prues works with Dr. Smith providing technical support and expects to accomplish the following:

My focus is to continue to assist in the research being conducted at the NMRI/TD providing my technical expertise as required. For the upcoming quarter that involves working with Dr. James Lindsey on his TMPP research as well as assisting Dr. Eldon Smith in his preparative work for the upcoming SFE project.

Naryanan, T.K., Jung

Our objectives for the next reporting period are to increase our productivity in the lab and continue the studies on separating and analyzing the metabolite(s) of DBNP, the TMPP binding studies, and continuing the work on the cell model project and the neurotransmitter project.

Ritchie

Dr. Ritchie expects to accomplish the following in support of the NMRI TOX/DET's neurobehavioral program:

NTAB Development:

- Complete and submit major new Work Unit, as described above.
- Complete juvenile play analysis.
- Complete rat Progressive Ratio testing.
- Complete Porsolt Forced Swim Test.
- Complete Pigeon Spectral and Geometric Pattern Analysis.
- Begin rat Shock Avoidance testing.

Ozone Depleting Substance Replacements:

- Complete two (2) months testing of R-134a, R-12 and halon-1211 on Progressive Ratio with rats and Spectral Discrimination tests with pigeons.

Single and Mixed Combustion Gas Atmospheres:

- Begin CO operant testing of pigeons.
- Begin CO2 roto-wheel testing of rats.
- Begin CO2 operant testing of pigeons.

TMPP:

- Coordinate TMPP behavioral teratology study with BGSU.
- Complete TMPP "absence epilepsy" study.
- Assist Dr. Jan Lin in scientific orientation and lab set-up.

Gulf Warfare Syndrome:

- Complete shocker/harness development.
- Begin initial animal exposures.
- Test control animals on neurobehavioral tests.

Ademujohn

Ms. Ademujohn works with Dr. Ritchie in the NMRI TOX/DET's neurobehavioral program and her goals for this next period includes:

- To accurately and efficiently compile, log, organize and analyze all incoming data from inhalation studies.
- To accurately train rodents for various testing protocols.
- To accurately train pigeons for upcoming testing protocols.
- To maintain a clean and orderly laboratory environment.
- To provide technical assistance in modified Wahmann chamber studies.
- To provide technical support in testing relative toxicity of CO, R134a, Halon 1301.
- To provide technical support in streamlining operant training methods for upcoming pigeon and rodent training protocols/testing.
- To procure and document pigeon maintenance pertaining to preparatory requirements for pre-testing or "shaping" activities.

Martin

Ms. Martin's objectives as technical editor for next quarter are to assist NMRI/TD and GEO-CENTERS's scientists with their conference presentations, to complete the reservist package, to update the research files, to reconfigure the publications files, to incorporate the project management process into the publications process, and to continue performing the duties outlined her position description. She will also present a poster at the NEHC conference and two posters at this year's April Conference. In addition, she will help prepare the Tri-Service Toxicology display booth for the Society of Toxicology conference and this year's April Conference as well.

Binole, Rix

In the next quarter, the ADP team at NMRI TOX/DET will continue to implement those software and hardware products which increase automation and productivity. Projects scheduled for the coming quarter include: (1) Upgrade of main servers to NTAS 3.5; (2) Conversion of Library and old main server to NTAS 3.5; (3) Install SMTP server dameon for NTAS to free us from the Air Force VAX; (4) Expand our internet capability by adding WAIS databases; (5) Continue to provide technical support for TOXDET personnel; and (6) Develop support software where needed.

Connolly

Ms. Connolly expects to continue her data informational services in the following ways:

- Continue cataloging.
- Continue providing service to the toxicology community at WPAFB.
- Continue sorting through reprint collection to eliminate duplication of articles on the shelves.
- Continue as a member of base level IPT on Libraries and Information Centers

Walsh

Ms. Walsh's goals for this next period in support of the Armstrong Toxicology Laboratory includes:

Inhalation Toxicity of Tricresyl Phosphate Vapor Phase Lubricant:

This study will continue with an exposure to blend of 85% 2 cSt PAO and 15% Tert Butyl Phenyl Phosphate. A second exposure to air only will be performed.

Combustion Toxicity of Advanced Composite Materials:

This study has been completed to date. With the data analysis of the B2 combustion completed it may generate additional questions which need to be addressed, thus generating additional work. The F22 material has yet to be analyzed and may very well begin this next quarter.

In Vitro-In vivo Extrapolation:

I will perform 10 additional liver perfusions. This will allow for perfection of the liver perfusion technique and additional analysis to be collected.

Dong

Dr. Dong will conduct the following chemical skin absorption studies during the next period:

- To measure CPFB and DCB absorption in hairless guinea pigs which resulted from dermal exposure of the two chemicals as soon as the hairless guinea pigs become available.
- To continue to search for the candidate of the third chemical for dermal penetration studies should perfluorohexyl iodide be dropped out.

Grabau

Dr. Grabau expects to accomplish the following

Species Differences in Skin Penetration:

- Initiate evaluation and development of image analysis algorithms to quantify immuno-histochemical markers of early events in chemically-induced skin inflammation.
- Present posters at the 34th Annual Meeting of the Society of Toxicology and the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995).

Combustion Toxicity of Advanced Composite Materials (ACM):

- Further evaluate results from the recently completed ACM combustion studies and plan follow-up *in-vivo* experiments.
- Coauthor posters to be presented at 34th Annual Meeting of the Society of Toxicology (March 1995) and the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995).

Trichloroethylene (TCE) Biologically Based Health Risk Model:

- Further image analysis will be conducted to quantify subcellular (PCNA, P450 isoenzyme and other markers) changes in liver cells exposed to TCE.
- Coauthored posters to be presented at the Society of Toxicology Annual Meeting (March 1995) and the Conference on Risk Assessment Issues For Sensitive Human Populations (April 1995).

Intra-Laboratory Research (ILR)Grant - Hepatic Apoptosis in Mice and Rats:

Develop and conduct image analysis on tissue collected from 100 rats. Develop and prepare to conduct image analysis on tissue from 200 mice to study apoptosis that will be available in July 1995.

Other Tasks:

- CRDA - Negotiations are expected to continue and become tangible results are expected in this quarter. This project has significant potential.
- Consultation for Tri-Service Marketing - Progress toward an interactive CD-ROM and video educational/market/public relations tape will continue.
- Coordination of Image/Presentation Resources - Coordination of computer-based graphic aids, research-associated photographs and presentation aids is expected to continue.

- Consultation for Laboratory Computer Requirements - A trip to a pathology GLP compliant software users group has been scheduled for 19-23 March 1995.

Geiss

Mr. Geiss expects to support the Armstrong Toxicology laboratory in Dayton via the following goals during the next reporting period:

- To complete analysis of tissue samples from the 60-day TCE study with histone, glyceraldehyde-3-phosphate dehydrogenase, and gamma-glutamylcysteine synthetase probes.

- Develop assays for other probes of interest, such as protein kinase C-alpha and ornithine decarboxylase.

- Design and synthesize oligonucleotides for use in analysis of mRNAs in samples from other projects.

- Complete next group of planned experiments for the hyperbaric oxygen project.

IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND ARTIFICIAL BLOOD STUDIES

Li

Dr. Li will continue to support the wound repair program at NMRI by anticipating the following achievements for the next period:

- Continue to study the effects of cytokine oral administration on mice HS model.
- Investigate the effects of IL-6 and CAS on the protection of physiological intestinal movement, especially the intestinal vaso-dilation function following HS.

Fan

Ms. Fan's molecular biology laboratory at NMRI will be focused on the following during the next quarter:

- Investigate the effect of LPS on PKC mRNA transcription and identify the 8 distinct PKC isotypes by RT-PCR and Southern blot, eventually sequencing of the PCR products.

- Continue colaboration with Dr. Volgo to further investigate gene regulations of cytokines in immune system of CLP-treated mice.

- Develop a new quantitative PCR technique using site-specific mutagenesis with asymmetric PCR and a single mutant primer.

Chavez

Dr. Chavez's program on extending the life of whole blood and blood components for NMRI and WRAIR is anticipating accomplishing the following during the next reporting period:

The pilot plant is now fully operational. My duties will focus on the research aspects during this quarter. The heme association experiments will be completed and the data will be prepared for publication. In April (date not known yet), a poster presentation will be done at WRAIR. For the next research project, a collaborative investigation with Dr. Victor Macdonald involving a heme exchange experiment with partially oxidized hemoglobin is on the agenda. Hemoglobin exists as a mixture of the oxidized and reduced form *in vivo*. In the oxidized form, hemoglobin has a much greater tendency to lose its heme than in the reduced form. This is presumably due to the reduced affinity of the heme for the proximal histidine of hemoglobin. Also known is that the protein conformation is different for the oxidized form versus the reduced form of hemoglobin. Characterization of the effects of the protein configuration on the heme loss will be accomplished.

Submission of an NIH Grant proposal is planned to support my research efforts and, if funded, provide technical personnel support. The proposal deadline is 1 June 1995. Mechanisms as to how to submit a grant proposal as a civilian contractor will be thoroughly investigated to avoid potential administrative pitfalls.

Dependent on other time requirements, the following experiments are still in the queue:

- One-step single column protein purification.
- Effect of NO binding to heme stability.
- Near-infrared studies on the hydration of hemoglobin.
- Fluorescence monitoring of the $\alpha\beta$ interface.
- Calorimetric determination of the tetramer -> dimer rate constant.

Ring

Dr. Ring's computer skills will be focused on the bone marrow research program at NMRI for the next reporting period:

- Improve and upgrade the mice tracking system.
- Develop, improve and upgrade other software programs as needed.
- Continue to expand the connectivity of the department.
- Collaborate with investigators from NIH in calcium imaging experiments. The collaboration is to be coordinated by Dr. June.

DENTAL DISEASES-RELATED RESEARCH

Turner

Dr. Turner will continue to provide NDRI with senior scientist level research by providing the following services:

Use of the fluorescent spectrophotomers will be learned. Some fluorescent dyes will be tested to determine which will work the best for us and the factors we plan to examine. Healthy and diseased subjects will be chosen for the study and their informed consent for inclusion in the study will be obtained from them.

Spencer

Ms. Spencer will continue to support NDRI's clinical research program by providing the following:

- Continue management and data collection for AO study.

- Assist in coordination of the Northwestern University study site.
- Assist on the amalgam waste water project with Dr. Roddy.
- Assist in the maintenance of the Tissue culture lab.
- Continue enlisted personnel training for promotion exam.

Lamberts

Dr. Lamberts will continue to revise and edit the manuscripts of the scientists at NDRI as follows:

- Complete the revision of the manuscript on immune response indicators in saliva as required for publication.
- Complete preparation of the microbiological manuscript from data of Dr. Simonson.
- Assist NDRI investigators editorially whenever requested.

Ahlf

Ms. Ahlf will provide dental research hygiene research at NDRI via the following goals:

- Complete the video tape on tobacco cessation.
- Assist in the completion of the Dental Examination Periodicity project.
- Discover the best way to accomplish the task of creating a tobacco cessation program for the recruit environment.
- Provide preventive treatment for NDRI command and establish appropriate maintenance schedules.
- Schedule follow-up sessions with the enlisted personnel supporting their advancement intentions.
- Complete the four day TQL training sessions.
- Enhance my lifestyle by incorporating PT into my schedule on a regular basis.

Miller

Dr. Miller plans to continue his senior scientist support to the NDRI Detachment at Bethesda by pursuing the following goals:

Work Unit: 0601152N.MR00001.001-0063. Evaluation of the influence of superantigens and polyclonal B-cell activators in periodontal disease To continue work on this Individual Research Program specifically focusing on the rt-PCR and flow work.. Attempts will also be made to begin

purification and characterization of superantigens and immunomodulators associated with periodontal pathogens.

Work Unit: 0601152N.MR00001.001-0063. IL-1 production by polymorphonuclear leukocytes resident in periradicular lesions. To continue development of an in-situ hybridization procedure to detect cytokine mRNA.

Work Unit: 0601152N.MR00001.001-0063. Long term frozen storage of lymphocytes. to complete the second part of this program with the quantitation of cytokines. It should be noted that enough lymphocytes remain frozen in nitrogen storage to permit us to evaluate activity several years from today.

Work Unit 63706N.M0095.006-3014. Influence of growth factors on gingival and periodontal ligament fibroblasts. A main goal will be to complete writing of the manuscript.

To initiate a new program designed to study lymphocyte heterogeneity in periradicular tissue. It is anticipated that approval from the SRC and from the Human Research Committee will be available by late spring or early summer.

Gu

Ms. Gu works with Dr. Miller and plans to accomplish the following during the next report:

To continue the major project: the determination of T-lymphocyte Vb mRNA.
- To complete PCR and gel with the 49 samples.
- To complete analysis and quantify of the 49 samples.
- To continue collaborative work with Dr. Joel L. Schwartz on oral cancer project

Solanki

Ms. Solanki works with Dr. Miller and provides technical services as follows for the nest reporting period:

Complete all required ELISA's to measure various cytokine activities for the effects of freezing of T cells experiments.

Proceed with "Influence of T. Denticola on cell proliferation" study and start evaluating cytokine activities by ELISA on supernatant of cells exposed to various antigens.

Continue with experiments for better protein separations and then separate bioproteins of crude bacterial prep successfully.

Continue with "Generations of Superantigen stimulated T cells for identification of VB positive cells" using PCR and flow cytometric procedures as more samples of stimulated lymphocytes are required to survey the nucleic acid messages.

BIOMEDICAL DIVING RESEARCH

Shea

Dr. Shea's support of the NMRI's diving medicine department will be to continue the experiments of the effects of NMDA lesions in the NBM. Both short and long term effects will be addressed. A true sham lesioning procedure will be used on the contralateral side of the brain.

The effects of glucose will be further examined in animals exposed to cold. This time i.p. injections will be used as this procedure was shown to reduce temperature effects on memory in a behaving animal.

Purchase of a nitric oxide *in vivo* instrument.

Kerr

Mr. Kerr works with Dr. Shea in support of the NMRI diving medicine program and will accomplish the following during the next period:

ALZHEIMER STUDY - The objectives for the next quarter include the following:
- Finish the lesioning portion of this study with an accurate time-course to lesion formation, and to answer the question, "If induction of B-APP occurs, when does it happen?"
- Write an abstract on this portion of the study for Neuroscience (due 5-1-95).
- Upon completion of lesion study, begin the amyloid study.

DIVING PHYSIOLOGY - The objectives for the next quarter include the following:
- Assist in finishing chamber modifications and begin the oxygen studies.
- Submit a technical report on oxygen/microdialysis techniques if chamber is ready.
- Write an abstract for Neuroscience on this study if enough data and results have been compiled (due 5-1-95).

Porter

Mr. Porter anticipates to complete the following during the next quarter at NMRI:

- To continue analysis of fleet soda lime for contaminates and dye concentration as needed.
- To continue testing program for candidate CO2 analyzers for both the dry deck shelter and sub ambient air programs.

Ruby

Mr. Ruby's work at NMRI will continue the testing of the candidate analyzers for the collaborative work with the NSMRL project and provide periodic reporting of the progress.

Continue the development of the NAVSEA candidate analyzers and the divers air bank sampling procedures.

Support the needs of the diving medicine functional areas as required.

PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

Salander

Ms. Salander will continue to train animals to perform within the NMRI thermal stress research program. She expects to accomplish the following:

- Continue training new rats on the FI/FR schedules of reinforcement.
- Once performance is stabilized in these rats under the multiple schedule, chronic cannula will be surgically implanted into the lateral ventricle in the brain.
- Begin a study to better understand the effects of stress, using CRF injections incorporating the use of blood analysis in particular, looking at corticosterone levels, and observing behavior.
- Begin a study to investigate the effects on blood flow in the rat tail when a reinforced schedule has been learned and stress through shock has been introduced.
- Begin training 6 rodents to successfully stay in a restraining device for 20min. after an injection of NPY, and record and analyze the effects on blood flow.

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2. The Defense Technical Information Center received the enclosed report (referenced below) which is not marked in accordance with the above reference.

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N00014-95-D-0048---1 DEC 94-28 FEB 95
TITLE: RESEARCH ON NAVY-RELATED
COMBAT CASUALTY CARE ISSUES,
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INJURIES AND ILLNESSES /APPROACHE

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Debra T. Hughes

DEBRA T. HUGHES
DEPUTY DIRECTOR
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(Signature & Typed Name) (Assigning Office)

25 SEP 96

(Date Statement Assigned)